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Expanding a classic woodland food chain into a geographically variable food web

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Thesis abstract

There is ample evidence that climate change is impacting on phenology and it has been suggested that this may generate trophic mismatches. A key system for investigating phenology and trophic mismatch occurs in spring in temperate deciduous woodlands, where folivorous caterpillars and their predators, insectivorous passerines, are reliant upon ephemeral resources for reproductive success and survival. However, studies are primarily conducted within single-site, oak- (*Quercus* sp) dominated woodland and focus on a single caterpillar species, winter moth (*Operophtera brumata*), despite these passerines being habitat generalists with large geographic ranges. It remains to be seen whether insights gained from these studies can be generalised on the landscape scale across different habitats. In this thesis, I explore the extent to which geographic and habitat variation operates in this system and attempt to expand the system beyond a linear single-species food chain into a more biologically realistic multi-species food web. I also identify the most important environmental factors predicting the phenology of the passerines to allow better predictions of how their phenology could alter under future climate change scenarios. To address these questions, I established a novel 220km transect of Scotland incorporating 40 field sites that vary in elevation and the type of deciduous woodland habitat, monitoring six blue tit (*Cyanistes caeruleus*) nestboxes, tree and invertebrate phenology and abundance, at each site throughout the springs of 2014-16.

Firstly, I assess how blue tit occupancy and productivity are affected by the variation in fine-scale woodland habitat, latitude, elevation and prey availability that exists along the transect (Chapter 2). I find that habitat variables strongly affect fledging success but not occupancy or clutch size, whilst occupancy exhibits biogeographic trends, revealing that the relationship between breeding decisions and outcomes differs among habitats and implies that it may be difficult to generalise results from one habitat to others.

Next, I aim to identify the environmental aspects which play a role in regulating blue tit reproductive phenology by examining the ability of temperature, tree phenology, invertebrate prey abundance and photoperiod to predict nest initiation and laying dates (Chapter 3). I find that night-time temperature in early spring is the most important predictor of both nest initiation and lay date (slopes ~ -3days/°C) and I suggest that this supports the hypothesis that temperature acts as a constraint on timing rather than a cue. Invertebrate abundance is also a positive correlate of lay date, possibly allowing fine-tuning of timing.

This knowledge provides clearer foundations from which to predict future phenological change and possible trophic mismatch in this system.

There is the potential that the apparent effect of temperature on blue tit reproductive phenology is indirect and mediated by diet, which is largely undescribed in the period prior to breeding. Therefore, in Chapter 4 I examine how blue tit diet varies across habitat, geography and time, and whether there is a dietary cue utilised to initiate breeding phenology, using data from metabarcoding faeces collected from nestbox-roosting adults in early spring. Geographic variation in diet is substantial, with high site-to-site dietary turnover (β-diversity), as well as high turnover along the elevational and latitudinal gradients studied. Dietary α-diversity (richness) is unaffected by geographical variables, but increases over time, with significant pre-breeding dietary increases in *Lepidoptera* and *Hemiptera* signifying a possible cue. In addition, these data provide the most comprehensive next-generation insights into the diet of a wild bird to date and identify 432 prey taxa.

Finally, I analyse how biogeographic and habitat variables affect the phenology, abundance and diversity of caterpillars (Chapter 5). Host tree species' varied significantly in their likelihood of hosting a caterpillar, with oak and willow (*Salix* sp.) the most likely. Biogeography had less effect on the likelihood of caterpillar occurrence, but elevation delayed peak date by 3.7 days/100m increase. There was also support for the spring caterpillar peak being dominated by a few key species, with over half of all caterpillars identified being of just three of the 62 total species, including winter moth. These findings contribute to understanding how the temporal distribution of caterpillars varies across habitats on the landscape scale.

Taken together, the findings of this thesis reveal considerable geographic and habitat variation throughout this system, in both the composition of the food web and the impacts on blue tit productivity, demonstrating why caution must be exercised when extrapolating findings from one location or habitat to others.

Lay summary

Climate change is causing a change in the timing of natural spring events, such as the leafing of deciduous trees, the emergence of the caterpillars that eat these leaves, and the breeding of the birds that eat the caterpillars. Whilst the trees and the caterpillars seem to be advancing their timing at the same rate, there is evidence that the birds are not advancing their timing quick enough to keep up, and are suffering reduced breeding success as a result, which could lead to population declines. Most studies have been conducted at single sites in Western Europe's climax vegetation structure, mature oak woodland. However, this vegetation structure is quite rare and the majority of the bird species in question, such as blue tits, live in a wide range of habitats and geographic locations. In order to see how much of an issue this is for them in general, we need to study this over more diverse habitat types and locations. Therefore, in this thesis, I aim to quantify how much geographic and habitat variation exists in this study system. To study this, I set up 40 field sites that varied in their elevation and woodland type across a 220km route through Scotland, and monitored what happened in six blue tit nestboxes at each site throughout the springs of 2014-16, as well as monitoring when tree leaves came out and how much prey was available for the blue tits.

Firstly, I analyse how woodland habitat, latitude, elevation and prey availability effects where blue tits choose to breed, how many eggs they lay and how successful they are at rearing their chicks through to fledging (Chapter 2). I find that whilst habitat doesn't seem to influence where blue tits breed and how many eggs they lay; it has large effects on fledging success. Where blue tits breed is more affected by geography, with fewer nesting at higher elevations and the further north one goes. This difference between the factors affecting breeding decisions and those affecting breeding outcomes implies that extrapolating results from one habitat to others may be difficult.

Next, I try to identify how blue tits time their breeding and the aspects of the environment that they are responding to - looking at temperature, tree leafing, prey abundance and photoperiod (Chapter 3). I find that night-time temperature in early spring is the best predictor of both when nesting begins and when the first egg is laid, with both advancing by about 3 days per 1°C increase in night-time temperature. Prey abundance also effects when eggs are laid and may allow for fine-tuning of timing. These results increase our ability to predict how timing will change in the future, and therefore its knock-on effects.

In Chapter 4 I identify adult blue tit diet in spring by looking at the prey DNA contained within their faeces. I find that geographic dietary variation is very large, showing that what the blue tits are eating is very different in different locations. In addition, the amount of different prey types contained in each faecal sample increases throughout the spring, in particular moths and aphids before breeding, which may show that these dietary items could be used by the blue tits as a signal to schedule breeding. These data also provide the most in-depth study of a wild bird's diet to date, identifying 432 different prey items.

Lastly, I look at how the amount of caterpillars, and their timing in spring, varies geographically and on different tree species, as well as identifying which species of caterpillars occur to form the food peak that is so important for the birds (Chapter 5). Oak and willow had the highest levels of caterpillars associated with them and increasing elevation delayed the caterpillar peak by almost four days per 100m of elevation. Just three species of caterpillar, out of 62 total species found, formed over 50% of all those I sampled and I infer that these are the most important for the birds. These findings contribute to understanding how the timing of caterpillars varies across habitats and in the wider landscape, which will effect when the birds should breed at each place.

Overall, the findings presented in this thesis show that there are high levels of geographic and habitat variation acting on the tree – caterpillar – bird system, both in terms of which species are involved in different locations and the impact that this has on the birds. Therefore, I suggest that care is needed when taking findings from one habitat or location and using them to infer what is happening at another.

Declaration

I composed this thesis with guidance throughout from my primary supervisor, Albert Phillimore. My secondary supervisor, Jarrod Hadfield, provided the initial idea of a transect and aided with the statistical framework and my tertiary supervisor, Malcolm Burgess, contributed comments and methodological ideas.

The work described in this thesis was carried out by me, or as acknowledged below.

Analyses performed throughout this thesis utilised data collected by myself, my primary supervisor and our field assistants: Irene Benedicto Cabello, Margaret Bolton and Edward Ivimey-Cook.

Chapter 2: This chapter was prepared as a manuscript; I wrote the first draft and co-authors contributed to subsequent drafts. Reviewer comments were received and addressed.

Chapter 4: Faecal metabarcoding labwork (section 4.3.2) was performed by James Nicholls, who also devised and prepared the labwork protocols provided in sections C1 and C2 (part of Appendix C). Initial bioinformatics (section 4.3.3) were performed by Urmi Trivedi at Edinburgh Genomics.

Chapter 5: Caterpillar DNA extraction and sequencing was provided by the Barcode of Life Database (BOLD) based at the University of Guelph, Canada.

This work has not, in whole or part, been submitted for any other degree or professional qualification.

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Landowners and managers along the transect:

Atholl Estates
B & H Sporting
Mr A Barbour
Mr P Barr
Mr J Batty
The Bein Inn
Berthapark Farm
Bowley's Farm
Mr G Brockman
Mr A Christie
Dalnacardoch Estate
Major I Dalzel-Job
Dundas Castle Estate
Laird A Findlay
Fordell Firs Scout Centre
Forestry Commission
Ms P Freeman
Ms E Garty
Mr R Hannigan
Mr G Leggat
RSPB Loch Leven
Mrs A MacLeod
Mrs D McBride
Mr A McKenzie
Mr D McKenzie
The Honourable P Moncrieffe of that Ilk
Mr A Munro
Mr H Munro, Chief of the Clan Munro
The National Trust for Scotland
Novar Estate
Ralia Enterprises
Rothiemurchus Estate
Mr R and Ms M Scobie
Scottish Natural Heritage
Seafield Estate
Speyside Distillery
Mr P Voysey
The Woodland Trust
Mr J and Mrs M Yule

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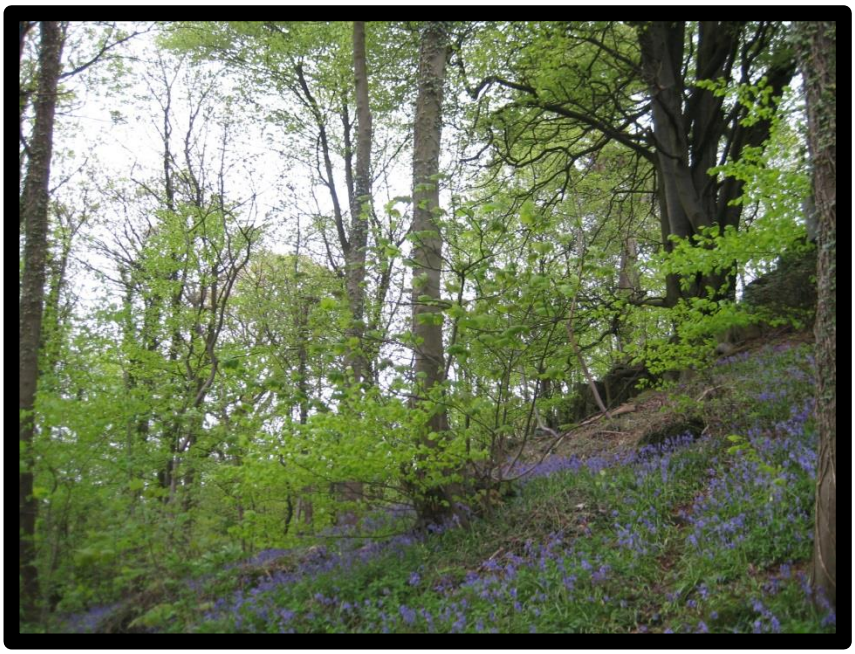
Chapter 1

General introduction



Fordell Firs

1.1 Climate change and phenology

Anthropogenic climate change is now a well-recognised and unequivocal climatic effect, with the 5th IPCC report stating that the world has warmed by 0.12°C on average per decade since 1951 and warming is expected to continue and increase in the decades to come (IPCC 2013). This changing climate is altering the abiotic environmental conditions experienced by organisms and leading to ecological effects including species distribution shifts, and phenological shifts (Walther *et al.* 2002; Parmesan 2006; Post *et al.* 2009). Phenology is the study of annually recurring life cycle events and their timings (Morren 1853; Visser *et al.* 2010); these events are often seasonal and examples include the timing of caribou (*Rangifer tarandus*) calving (Post *et al.* 2003) and the annual mass emergence of certain mayfly (*Ephemeroptera*) species (Everall *et al.* 2015). Species often utilise changes in ambient temperature to coordinate timing and thus phenological events are a highly sensitive ecological indicator of climate fluctuations (Walther *et al.* 2002; Edwards & Richardson 2004). The advancement of spring phenological events in the northern hemisphere across all taxa currently averages 2.3 days advancement per decade (Parmesan & Yohe 2003) but with substantial taxonomic variation (Thackeray *et al.* 2016).

The timing of phenological events impacts on fitness and therefore there is likely to be selection for synchrony with optimal environmental conditions, which could be abiotic (e.g. temperature, rainfall) and/or biotic (e.g. prey resource availability, predator avoidance) (Darwin 1859; Visser *et al.* 1998; Miller-Rushing *et al.* 2010). The costs of mistiming can include reduced reproductive rates (Floate, Kearsley & Whitham 1993; Post & Forchhammer 2008) and offspring survival (Pearce-Higgins, Yalden & Whittingham 2005; van Asch & Visser 2007; Plard *et al.* 2014) and have been observed across many taxa. If all members of a population are mistimed the mean fitness may be depressed and this can cause demographic effects including population declines (Both *et al.* 2006; Willis *et al.* 2008) and local extinctions (Winder & Schindler 2004; Singer & Parmesan 2010). Phenology also governs species interactions in time, thereby impacting food webs and thus the fitness of individuals of other species (Miller-Rushing *et al.* 2010). Consequently, phenology is of interest because it is an indicator of climate change, a determinant of species interactions, and impacts individual fitness.

A greater understanding of how phenology operates and its subsequent effects on fitness and species interactions within particular ecosystems is required in order to predict how

individuals, populations, species, and communities, will respond under future climate change scenarios (Crick & Sparks 1999; Edwards & Richardson 2004). More specifically, greater insights are required into how phenological events are timed, what environmental factors predict them and how these predictors of phenology differ between interacting species. This knowledge will allow greater confidence in predicting future phenological changes and in turn will highlight particularly susceptible species, communities and ecosystem services (e.g. predation of forestry pests, maintenance of fisheries) and possibly enable mitigation attempts for any seriously deleterious predicted consequences.

Food chains in temperate deciduous woodlands have become a model system for studying many biological principles, including phenology, with the deciduous tree – folivorous caterpillar – insectivorous passerine bird food chain the focus of many studies (Visser *et al.* 1998; Charmantier *et al.* 2008). This system lends itself to phenological study as it is driven by a highly seasonal environment and abundant food resources are available for short time periods, thereby making phenology critically important for fitness across multiple interconnected trophic levels. Temperate deciduous trees produce fresh leaves en-masse each spring and must time this correctly to avoid both damage from freezing temperatures (if they produce leaves too early) and missing valuable sunlight resources, reducing their competitiveness (if they produce leaves too late) (Linkosalo *et al.* 2000; Vitasse *et al.* 2011). These fresh leaves in turn support large populations of folivorous caterpillars of many *Lepidoptera* species which must time their emergence to coincide with the fresh leaves for increased fitness (Feeny 1970; Hunter 1990); emerging too early reduces food availability and if they emerge too late the trees have released unpalatable tannins and other defensive chemicals into their leaves, reducing caterpillar growth and survival (Forkner, Marquis & Lill 2004; Forkner *et al.* 2008). This creates an ephemeral superabundance of prey for insectivorous passerines such as tits (*Paridae*) and flycatchers (*Muscicapidae*), which rely on reproductive synchrony with this annual bonanza to feed their nestlings and increase productivity (Wilkin, King & Sheldon 2009; Burger *et al.* 2012), with most caterpillar-feeding species being predominantly single brooded (Perrins 1979; Lundberg & Alatalo 2010). This study system will provide the focus for this thesis.

1.2 Environmental predictors of phenology

To make predictions about how phenology and dependent interactions could change in future, it is valuable to have a good understanding of the environmental factors that

determine timing and the mechanism whereby the individual responds to the environment (Vitasse *et al.* 2009; Caro *et al.* 2013). For example, identifying which environmental cues the organism is responding to and/or physiological constraints they are restricted by, and determine the timing of the phenological response. Where environmental variables deliver reliable information about the future environment, this information can be used by organisms to schedule phenological events under beneficial environmental conditions. However, a predictor that correlates with optimality in evolutionary history does not necessarily infer optimality in future if the predictor used loses correspondence with the intended timing (Visser *et al.* 1998; Phillimore *et al.* 2016). Such a situation is likely under climate change situations where environmental conditions shift and diverge (Durant *et al.* 2007; Gienapp, Reed & Visser 2014).

Directly assessing the mechanisms underpinning the use of environmental predictors of phenology is extremely difficult. The most common approach has generally involved regressing phenological dates on potential environmental predictors (Slagsvold 1976; Sparks & Menzel 2002; Källander *et al.* 2017). If the environmental variable significantly predicts the phenological events, a population-level reaction norm can be determined, whereby a change of x in the predictor variable elicits a response of y days advance or retreat in the date of the phenological response. This approach is also amenable to application of a sliding window approach, whereby the effects of a focal environment on a range of dates can be regressed against phenological dates to determine the period over which the environment has the strongest, or most significant, effect upon the phenological event (Husby *et al.* 2010; Phillimore *et al.* 2016).

A frequently proposed environmental predictor in many systems is mean temperature, or increasing temperature, over a sensitivity window (a period of time wherein the organism senses temperatures and is responsive to them) (Both *et al.* 2004a; Schaper *et al.* 2012). Phenological responses to temperature could be driven directly as a cue sensed from the changing temperature (Visser, Holleman & Caro 2009), or as a constraint via a thermal limitation to physiological processes (Stevenson & Bryant 2000). Alternatively, the effect of temperature could be indirect and the proximate cause is a correlated factor, such as food availability or environmental phenology (Thomas *et al.* 2010; Bourgault *et al.* 2010). Distinguishing between these pathways from temperature to phenological response could also be important, as they may diverge from each other under climate change and predictions

of phenological change based on the direct pathway may differ from those based on an indirect pathway.

The key mechanisms determining deciduous tree leafing phenology in spring are relatively well established (Polgar & Primack 2011). Most temperate species appear to respond to temperature forcing leaf growth through the accumulation of growing degree days, whilst others also require chilling events over the winter (Vitasse *et al.* 2009; Roberts *et al.* 2015), or photoperiod (Zohner & Renner 2015), probably as safeguards to ensure that winter has passed before growing new leaves (Linkosalo *et al.* 2000).

The predictors of invertebrate phenology, such as *Lepidopteran* caterpillar emergence in deciduous woodlands in spring have received little attention. Many invertebrates require heat for growth (Buse *et al.* 1999; Petavy *et al.* 2001) and are thermally limited in their development (Bale *et al.* 2002). Winter moth (*Operophtera brumata*) caterpillars, often cited as the most numerous single species contributing to the spring caterpillar peak across much of Europe (Hunter & Willmer 1989; Wesolowski & Rowinski 2006a), emerge earlier and grow faster under warmer conditions (Buse *et al.* 1999). They seem able to track host tree leafing phenology both over time and within years and it has therefore been hypothesised that they respond to temperature over a similar timeframe to their host tree (Buse & Good 1996; van Asch *et al.* 2012). As the winter moth is a generalist, often feeding upon multiple tree species within a landscape (Waring & Townsend 2017), this response could be locally evolved to synchronise emergence with budburst of the most important local host (Wesolowski & Rowinski 2006a).

The environmental predictors of the breeding phenology of insectivorous passerine birds in this system are similarly unclear. The predictor used is unlikely to be directly connected to the required phenological timing, as the caterpillars that form the food peak with which synchronicity is important are not available in the environment when the birds need to initiate their reproductive phenology, as the birds require time for territory and mate selection, nest building and egg laying (Charmantier *et al.* 2008). This requires around a month in most species (Perrins 1970, 1996; Lundberg & Alatalo 2010). Consequently, an indirect environmental predictor that has broad correspondence to this future food peak is most likely. As there are multiple stages to passerine reproductive phenology, as discussed above, it is possible that multiple environmental predictors are utilised that enable 'fine-tuning' of timing throughout (Cresswell & McCleery 2003; Simmonds *et al.* 2017).

Photoperiod is known to be an important regulator of avian reproductive cycles away from the tropics (Lofts & Murton 1968), stimulating gonadal development, follicular growth and signalling song production (Dawson *et al.* 2001; Helm *et al.* 2013). However, most of these effects are triggered around the spring equinox in tits, long before reproduction (Caro *et al.* 2006), and also, as photoperiod is inter-annually consistent, it cannot explain inter-annual differences in an individual's timing, which are often substantial (Verhulst, Van Balen & Tinbergen 1995). Despite this, photoperiod can determine the wider time window during which egg-laying is possible (i.e. spring-time) and could explain between-population or individual timing differences if the evolved reaction norm differs between populations or individuals (Lambrechts *et al.* 1997b)

Temperature is also known to correlate with passerine reproductive phenology, with a 1°C rise during periods of early spring eliciting a 3.5-4.5 day advancement in tit clutch initiation (Visser *et al.* 1998; Phillimore *et al.* 2016). The means by which temperature affects the birds is unknown (Caro et al. 2013) and there is limited evidence to assess whether temperature is a direct cue or a constraint, or operates via an intermediary correlated variable. A possible intermediary variable could be tree phenology, which responds to temperature, and has been shown to correlate with tit reproductive phenology (Nilsson & Källander 2006; Hinks *et al.* 2015). However, studies purporting to show evidence for an effect of tree phenology often do not analyse other possible predictors simultaneously, and manipulating experienced tree phenology in aviary experiments is difficult and attempts at this have prompted no phenological response from tits (Visser *et al.* 2002; Schaper *et al.* 2011).

Another possible intermediary factor is food availability, and manipulating food availability can advance laying date, albeit only by a few days (Nager, Ruegger & van Noordwijk 1997; Robb *et al.* 2008a). Increasing prey abundance could either act as a cue, or as a release to a constraint, lifting the organism above the metabolic threshold level required to be able to begin the phenological event (Nager *et al.* 1997; Seward *et al.* 2014). The role of natural food availability and particular dietary items acting as a cue are unknown, but unlikely to be plant based chemical cues due to constant and very low levels of consumption (Bourgault, Caro & Perret 2006). Passerine diet prior to breeding is relatively poorly known and the taxonomic resolution of previous work has been too low to assess dietary drivers of reproductive phenology in these species (but see Betts 1955).

Our lack of knowledge of the predictors of woodland passerine reproductive phenology may in part be due to the difficulty of separating the effects of different environmental predictors on the scale of the majority of phenological studies to date: a single habitat at a single site. At this level, all of the putative environmental predictors, with the exception of photoperiod, are likely to positively co-vary across years. This lack of substantive evidence supporting predictors of woodland passerine reproductive phenology is a major issue and substantially limits the reliability of predictions of how phenology could change in future and what effect this will have on the ecosystem and at what scale. What is missing is a system under which the effects of the different predictors vary and can be teased apart, as well as a more accurate description of passerine diet, and its potential to act as a predictor at this time of year.

1.3 Trophic mismatch

1.3.1 The state of the art

If a phenological event is mistimed, for instance if the predictor used to time the event no longer corresponds to optimal timing, trophic mismatch can occur. Trophic mismatch theory was originally developed in marine fisheries (Cushing 1969) and postulates that annual variation in the fitness of a consumer is determined by the temporal coincidence between its phenology and the phenology of a prey species, or prey group, at the lower trophic level. A consumer mismatching their phenology (temporal asynchrony between their phenology and that of their prey) incurs reduced fitness (Edwards & Richardson 2004; Durant *et al.* 2007). If mismatching occurs for many individuals within a population, negative demographic effects and population declines or local extinctions could occur, with downstream effects on the whole ecosystem (Winder & Schindler 2004; Singer & Parmesan 2010). Consequently, the relative phenology of a focal consumer species with the phenology of resource species is often of more importance than absolute phenological timing (Miller-Rushing *et al.* 2010).

Whilst spring phenology is advancing by 2.3 days per decade on average in the northern hemisphere, this advance is not uniform within and between communities (Parmesan & Yohe 2003). Phenological shifts in response to climate change are determined by the degree to which a plastic response is possible using current environmental predictors, or local adaptation to the evolved response mechanisms (Phillimore *et al.* 2012; Charmantier & Gienapp 2013). Environmental predictors respond differently to increasing temperatures, and

as predictor use and plastic phenological responses are species-, or even population-, specific, dissimilar phenological responses to a temperature alteration are expected throughout an ecological community (Durant *et al.* 2007). Environmental predictor use that currently results in year-to-year synchronicity in phenological events among trophic levels may diverge and lose correspondence, temporally decoupling peak consumer requirement from peak resource availability (Figure 1.1). Even in the absence of differential predictor use, climate change should invariably result in selection on consumers due to their reaction norms being adaptively flatter than that of the resource due to the environment of development not being a perfect predictor of optimal timing (Gienapp *et al.* 2014). In general, secondary consumers in terrestrial ecosystems have been observed to show significantly less climate sensitivity and responsiveness than other trophic levels (Thackeray *et al.* 2016).

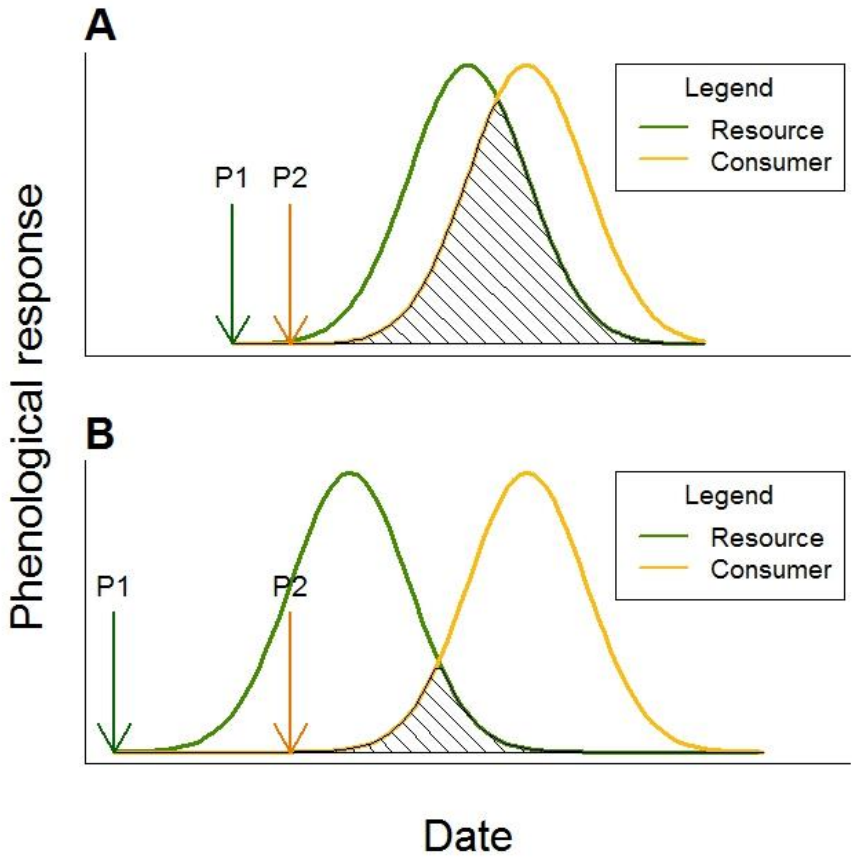


Figure 1.1 Schematic illustrating how two environmental predictors of phenology (P1 for the resource and P2 for the consumer) can result in phenological synchrony (**A**), but if they diverge trophic mismatch can ensue (**B**). The hatched area denotes the degree of overlap (match) between the phenology of the resource and that of the consumer, with the reduction in the size of the hatched area from A to B representing trophic mismatch.

In temperate woodland ecosystems, deciduous trees (the primary producers) are advancing their leafing phenology at a mean rate of around a week per 1°C increase (Sparks & Carey 1995; Vitasse *et al.* 2009). Folivorous caterpillars, the primary consumers in this system, are advancing their emergence at a similar rate and maintaining phenological synchrony with their food supply (Buse *et al.* 1999; van Asch *et al.* 2012). Insectivorous passerines, the secondary consumers in this system, are advancing their breeding phenology at around half this rate (Visser *et al.* 1998; Phillimore *et al.* 2016). Tits and flycatchers with greater proportions of caterpillars in their diet successfully raise both more and heavier fledglings and mismatching with this caterpillar peak has reduced their productivity (Visser, Holleman & Gienapp 2006; Wilkin *et al.* 2009; Burger *et al.* 2012).

Despite trophic mismatches reducing the individual fitness of passerines and reducing total fledgling numbers from a population in some years, there are often no observable effects on demography (Reed, Jenouvrier & Visser 2013b). Counterintuitively, populations of great and blue tits (the most frequently studied tit species) are actually stable or increasing throughout the UK and Europe (Blair & Hagemeijer 1997; Balmer *et al.* 2013). This is possibly due to a decoupling of productivity and demography, whereby an impact on productivity does not necessarily translate into a demographic effect (McLean *et al.* 2016). This situation might imply that density-dependent winter survival, rather than annual productivity, is the primary driver of demographic change in these species (Reed *et al.* 2013a), and winter survival has been increased by warmer winters and artificial feeding of birds in gardens (Balmer *et al.* 2013; Reed *et al.* 2013a). In the migratory pied flycatcher, conversely, populations are declining faster in geographic areas where trophic mismatch is stronger (Both *et al.* 2006). Therefore, the impact of trophic mismatch on demography could be population- or species-specific. Alternatively, mismatch may not yet have reached sufficient severity to provide noticeable population changes in tits, or areas outside phenological study sites may have negligible mismatch and be acting as source populations.

1.3.2 The limitations of current knowledge

A major limitation of trophic mismatch studies in this system to date has been the predominant and almost exclusive focus on oak-dominated mature woodlands as study sites. Whilst these habitats are considered to be the climax vegetation structure across much of Western Europe (Tansley 1939; Ozenda & Borel 2000) and we now know much about how trophic mismatch operates in them, they are an uncommon habitat (Forestry Commission

2013) and most woodland passerines are woodland generalists (Perrins 1979; Lundberg & Alatalo 2010) and therefore mature, oak-dominated habitats are unrepresentative of the average (and range of) habitats encountered by these birds. How basic productivity varies and trophic mismatch operates in other temperate deciduous woodland habitats is unknown. Without this knowledge, it is impossible to judge how important climate-mediated trophic mismatch is to passerine metapopulations (Cholewa & Wesołowski 2011; Burger *et al.* 2012). This is potentially of great import, as the negative effects of trophic mismatch in oak woodlands could be buffered on a landscape level if mismatch is less important, and/or initial productivity equal or greater, in other occupied habitats.

The vital middle link in the food chain, the caterpillars, is the least understood, and often neglected in trophic mismatch studies. This could be due to difficulties in data collection, with indirect frass (invertebrate excrement) collection the method most employed due to its ease (Visser *et al.* 1998; Smith *et al.* 2011). However, this is species- (and even order-) unspecific and questions remain regarding the exact species composition of the spring caterpillar peak and how this varies by location and/or tree species, as invertebrate communities are highly host-specific and vary between host tree species (Kennedy & Southwood 1984; Waring & Townsend 2017). Whether all caterpillar species have similar phenology and phenological predictors is poorly known (Veen *et al.* 2010) but could have important implications for trophic mismatch in this system which is currently unaccounted for. Whilst many caterpillar species are known to contribute to the spring caterpillar peak (Kennedy & Southwood 1984; Hunter 1992), winter moths are the only species invoked in the literature due to their apparent ubiquity and abundance, particularly in the commonly studied oak habitats (Wilkin, Perrins & Sheldon 2007). Winter moths are generalist feeders; however there is some evidence suggesting that they are found in varying abundances on different tree species and in different geographic situations (van Dongen *et al.* 1994; Wesolowski & Rowinski 2006a). This would affect the biomass of the resultant caterpillar food peak (Veen *et al.* 2010; Burger *et al.* 2012) regardless of phenology and, in caterpillar-poor habitats or locations, could exacerbate any deleterious mismatch effects endured by the birds purely due to a natural dearth of local food supply.

Lepidopteran caterpillars are the most abundant folivorous invertebrates in spring (Southwood *et al.* 2004) and are important to passerine productivity (Wilkin *et al.* 2009). However, tits and flycatchers are fairly generalist in their diets (Betts 1955; Cramp & Perrins 1993; Lundberg & Alatalo 2010), consuming many invertebrates and consequently focussing

solely on the importance of caterpillars may be unrealistic. Geographical variation in diet, dietary plasticity and the possibility of dietary shifts in response to mistiming reproduction with regards to caterpillars, have been under-studied. Dietary adaptability could provide a possible offset to the negative effects of mismatch and warrants the expansion of this study food-chain (oak tree – winter moth caterpillar – tit/flycatcher) to a more biologically realistic food web.

1.4 The value of spatial replication in phenological studies

The importance of trophic mismatch for insectivorous passerines on a landscape scale remains somewhat unclear due to the lack of standardised geographic replication in mismatch studies, with studies generally focussing at a very local level. Whilst this approach allows for accurate measurement at the local scale, single sites may be unrepresentative of productivity and mismatch trends in the wider landscape. Indeed, some local studies have revealed opposing insights into whether passerines are maintaining reproductive synchrony with the spring caterpillar peak and this highlights the requirement for a broader geographic view (Visser *et al.* 1998; Charmantier *et al.* 2008). Furthermore, geographic variables such as latitude and elevation may impact phenology and/or productivity (Fielding *et al.* 1999; Evans *et al.* 2009), and influence subsequent trophic mismatch. This could either buffer against population effects of mismatch, or increase their severity if the single sites currently studied underestimate patterns occurring in the wider landscape. This lack of geographic and habitat replication thus makes it unclear as to whether inferences gained at one site can be extrapolated to others.

The overwhelming majority of phenological studies are conducted at single sites. Some have used two or three geographically separate but close sites in attempts to compare phenology between habitats (Tremblay *et al.* 2003; Marciniak *et al.* 2007; Husby *et al.* 2010). Exceptionally, rare studies have gathered data from multiple independent locations and recombined them in meta-analyses which provide the greatest spatial replication in phenological studies to date, albeit non-standardised (Both *et al.* 2006; Burger *et al.* 2012). However, these studies are the exception and few phenology and mismatch studies across temperate woodlands are well replicated. A benefit of increased standardised spatial replication in phenological studies is that it would enable more generalised insights into phenological patterns and consequences occurring in the wider landscape and in different habitats and situations. Greater spatial replication would also permit the analysis of whether

geographic or habitat variables alter phenology and/or productivity and any subsequent levels of mismatch, and whether certain locations benefiting could offset the fitness costs incurred by other populations to stabilise metapopulations (Visser *et al.* 2003). Variance between the possible predictors of woodland passerine reproductive phenology among sites would also be greater and allow finer insights, disentangling predictors that often vary in a similar fashion at individual sites within a year (Figure 1.2).

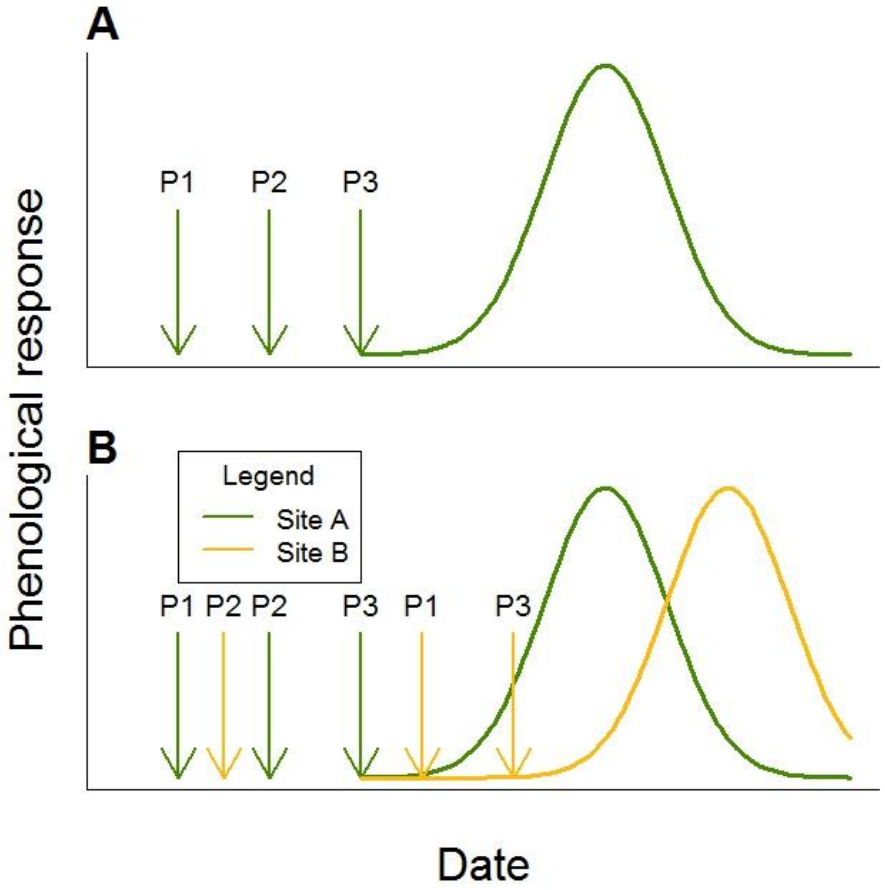


Figure 1.2 Schematic showing how the effects of three potential environmental predictors of phenology (P1, P2 and P3) are hard to separate at a single site (**A**) as they vary in a similar fashion, but may vary in a different fashion at a different site, with the comparison between the two sites allowing their differing effects to be disentangled (**B**).

One further advantage of higher spatial replication is the potential it offers for space-for-time substitution. This approach posits that if a species naturally occurs under a range of conditions, studying its phenology and responses in conditions experienced at site x (for example, a higher temperature) will provide insights into how the species will react at site y (where they currently experience cooler temperatures) if, over time, site y becomes more like site x (i.e. increasing temperature) (Dunne *et al.* 2004; Phillimore *et al.* 2013). In this way,

observations across environmental gradients (e.g. latitudinal, elevational and temperature gradients) can reveal phenological responses under varying environmental conditions, which could indicate projected future climates (Dunne *et al.* 2004; Hadfield 2016). A caveat of space-for-time substitution is that it assumes and requires that processes and patterns occurring over time are similar to those occurring across space in order to provide an informative surrogate. Whilst this assumption is logical, as processes that occur over space currently have also occurred over historical time (Phillimore *et al.* 2010, 2012), it may be untrue and provide false estimations of reactions over time. But, as long-term phenological time series are rare and require decades to attain (Sparks & Carey 1995), space-for-time substitution provides a time-effective alternative approach for evaluating phenological relationships and predicting future phenological reactions based on observations of phenological reactions from the past.

1.5 Thesis aims

The overarching aim of this thesis is to establish how geographic and habitat variability operates in a temperate woodland food chain and predicts phenology and productivity. It is hoped that this knowledge will form a baseline from which trophic mismatch at a landscape level can be explored in future and that this will aid in predicting how these communities will react to future climate change. I will expand phenological study in this well-established model system from a single-species food chain (oak tree – winter moth caterpillar – insectivorous passerine) studied at single sites into a more biologically realistic food web that can vary by location and habitat.

More specifically, I aim to assess the following questions primarily using blue tits as a model insectivorous woodland passerine:

i. Test how fine-scale deciduous woodland habitat, geographic factors and food abundance affect blue tit territory occupancy and two measures of productivity: clutch size and fledging success.

ii. Identify the key environmental predictors of blue tit reproductive phenology by disentangling the effects of temperature, photoperiod, tree phenology and prey abundance alongside exploring the possibility of a specific dietary cue.

iii. Using a novel faecal metabarcoding technique, I aim to provide a high taxonomic resolution description of blue tit diet and establish how dietary α- (richness) and β- (turnover) diversity vary over geographical, habitat and time gradients prior to reproduction.

iv. Determine how caterpillar community composition, biomass and temporal distribution vary by habitat, tree species and geographically, and estimate the contribution to the caterpillar peak made by winter moths and whether this varies at the landscape level.

To address these questions, I developed a unique 40 site, 220km transect across Scotland along a roughly north-south axis incorporating almost two latitudinal degrees and 450m of elevational gradient. The habitats of all sites were predominantly deciduous woodlands, but varied substantially in their constituent tree species composition, maturity and density. The situational variability along this transect allows for a greater understanding of phenological processes at the landscape level in this system than previous studies.

Chapter 2

The effects of woodland habitat and biogeography on blue tit territory occupancy and productivity along a 220km transect

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Glenfarg

2.1 Abstract

The nesting phenology and productivity of hole-nesting woodland passerines, such as tit species (*Paridae*), has been the subject of many studies and played a central role in advancing our understanding of the causes and consequences of trophic mismatch. However, as most studies have been conducted in mature, oak-rich (*Quercus* sp.) woodlands, it is unknown whether insights from such studies generalise to other habitats used by woodland generalist species. Here I applied spatial mixed models to data collected over three years (2014-2016) from 238 nestboxes across 40 sites that vary in woodland habitat and elevation along a 220km transect in Scotland. I evaluate the importance of habitat, biogeography and food availability as predictors of mesoscale among-site variation in blue tit (*Cyanistes caeruleus*) occupancy of nestboxes and two components of productivity (clutch size and fledging success). I find that habitat was not a significant predictor of occupancy or clutch size, but that occupancy exhibited pronounced biogeographic trends, declining with increasing latitude and elevation. However, fledging success, defined as the proportion of a clutch that fledged, was positively correlated with site level availability of birch, oak and sycamore, and tree diversity. The lack of correspondence between the effects of habitat on fledging success versus occupancy and clutch size may indicate that blue tits do not accurately predict the future quality of their breeding sites when selecting territories and laying clutches. There is little evidence of spatial autocorrelation in occupancy or clutch size, whereas spatial autocorrelation in fledging success extends over multiple sites, albeit non-significantly. Taken together, these findings suggest that the relationship between breeding decisions and breeding outcomes varies among habitats, and I urge caution when extrapolating inferences from one habitat to others.

2.2 Introduction

Temperate hole-nesting woodland passerines, such as tits (*Paridae*) and flycatchers (*Muscicapidae*), have become well used model systems for understanding trophic mismatch, specifically examining the effects of spring temperature on trophic interactions and fitness (Visser *et al.* 1998; Thomas *et al.* 2001; Both *et al.* 2004b; Charmantier *et al.* 2008). Most studies addressing trophic mismatch in these birds have been conducted in single-site mature woodlands dominated by a single tree species, usually oak (*Quercus* sp.) (Charmantier *et al.* 2008; Wilkin *et al.* 2009). However, many of these species are woodland generalists, occupying a wide variety of woodland types across their range and not all individuals within a population will experience similar environments. Therefore in order to extrapolate findings obtained in oak woodlands on a landscape- or meso-scale we first need to understand how habitat affects occupancy and productivity (Visser *et al.* 2003; Burger *et al.* 2012; Cole *et al.* 2015) as habitat can be a key determinant of fitness (Pärt 2001; Wilkin *et al.* 2007; Atiénzar *et al.* 2010). For instance, if a species is found to be most abundant and productive in oak woodland, by gaining an understanding of climate-mediated mismatch in this habitat we can better predict the metapopulation level impacts of mismatch. Alternatively, if habitats other than oak are found to benefit occupancy and productivity then we may also need to understand how mismatch operates in these different habitats.

Previous work examining the effect of breeding habitat on tit productivity has typically considered variation among territories at a single site (Perrins 1979; Wilkin *et al.* 2009; Amininasab *et al.* 2016) or between two or three sites (Blondel *et al.* 1991; Tremblay *et al.* 2003; Marciniak *et al.* 2007). For the two most frequently studied tit species, great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*), differences among major woodland types are widely documented, with clutch sizes and fledgling numbers approximately one third larger in deciduous compared with coniferous (Gibb & Betts 1963; Perrins 1965; Van Balen 1973) or sclerophyllous (Blondel *et al.* 1993; Lambrechts *et al.* 1997a) woodlands and breeding densities several times higher (Perrins 1979; Cramp & Perrins 1993).

Within deciduous woodlands tree species composition and maturity can vary substantially, though the effect of this fine-scale habitat variation on tit abundance and breeding performance has received little attention. Oak (*Quercus* sp.) is widely regarded to be the optimal breeding habitat for great and blue tits (Perrins 1979), with some studies defining territory quality on the basis of the number of oak trees they contain (Wilkin *et al.* 2007;

Bell, Owens & Lord 2014). In support of this assumption, great and blue tits forage more frequently in oaks when they are present than other tree species during the breeding season, but also visit a wide variety of other trees (Gibb 1954) and blue tit breeding densities and clutch sizes are higher in mature oak habitats than beech (*Fagus sylvatica*) (Amininasab *et al.* 2016). However, the relationship between the abundance of other tree species and tit breeding parameters remains largely unexplored, possibly a consequence of limited habitat variation within the typical single site study. A few studies have also examined the effect of other aspects of woodland composition and find that woodland maturity positively affects blue tit fledging success (Arriero, Sanz & Romero-Pujante 2006), whilst clutch size and occupancy are unaffected by woodland structure and management (Hinsley *et al.* 2002; Arriero *et al.* 2006; Burgess 2014).

On a mesoscale, as latitude and elevation increases, abiotic conditions such as temperature, rainfall and photoperiod may change, which in turn may affect habitat composition and food availability. Orell and Ojanen (1983) found no latitudinal trends in great tit clutch sizes across Europe whereas Sanz (1998) found that they lay marginally lower clutch sizes at the extremes of their European latitudinal distribution, a result corroborated in blue tits (Fargallo 2004), but that on the scale of country-wide latitudinal ranges these effects were very weak. Evans et al (2009) also found little evidence for latitudinal gradients in clutch size at a country-wide (UK) latitudinal range across a range of species, including tits. Increasing elevation has been shown to predict a small but significant reduction in the clutch size of great and blue tits (Sanz 1998; Fargallo 2004). While the mechanistic underpinnings of any relationship between these biogeographic variables and breeding parameters is unclear, if after controlling for local habitat such trends exist, this may imply either that the abiotic environment has a direct or indirect effect, or that habitat on a broader scale is important.

Food availability is one component of the biotic environment that may have profound impacts on geographic variation in species occurrence and productivity. Tits are mainly insectivorous during the breeding season (Betts 1955; Cholewa & Wesołowski 2011), and whilst they have been shown to rely heavily on an ephemeral peak in caterpillar abundance (Feeny 1970; van Dongen *et al.* 1997; Southwood *et al.* 2004) for provisioning of nestlings (Visser *et al.* 1998; Charmantier *et al.* 2008), at other times during the spring adult birds prey upon a broad range of additional taxa that includes flying invertebrates such as *Hemiptera*, *Diptera* and *Hymenoptera* (Betts 1955; Cowie & Hinsley 1988). Woodland invertebrate diversity and abundance varies considerably between tree species (Southwood, Moran &

Kennedy 1982; Kennedy & Southwood 1984). Given that different invertebrate taxa vary in their phenology (Niemela & Haukioja 1982; Southwood *et al.* 2004), the abundance and temporal availability of prey may vary in space (Fielding *et al.* 1999; Smith *et al.* 2011), which could affect productivity (Wilkin *et al.* 2009) and nest site selection decisions. Indeed, a positive effect of resource availability on productivity has been revealed via supplementary feeding experiments (Nager *et al.* 1997; Robb *et al.* 2008b), although this effect could be dependent upon the existing natural resource level (Bourgault, Perret & Lambrechts 2009).

The focus of this study is on identifying the effects of habitat and biogeography on blue tit occupancy and productivity. I aim to establish the relative importance of fine-scale woodland habitat versus food availability, and larger scale biogeography, as predictors of tit occupancy and on two components of productivity, clutch size and the proportion of the clutch that fledges. This knowledge will also help form a baseline from which to explore the complex mechanisms of trophic mismatch. I focus on blue tits, which are single-brooded woodland generalists that often exist in high density across Europe (Blair & Hagemeijer 1997; Balmer *et al.* 2013). This species is relatively sedentary, with natal dispersal probably of more importance to occupancy decisions than breeding dispersal at the scale I evaluate (Paradis *et al.* 1998). Rather than focusing on the effects of among territory habitat variation within a single site, this study considers among site habitat variation on a mesoscale. Specifically, I analyse data arising from a transect extending 220km in Scotland, incorporating 40 woodlands and spanning two degrees of latitude and almost 450m of elevation, encompassing a broad sample of habitats occupied by blue tits rather than focussing solely on large mature woodlands in order to provide a more representative sample of average blue tit habitat than previous work.

2.3 Methods

2.3.1 Transect study design

The fieldwork was conducted along a 40-site transect from Edinburgh (55.98°N, -3.40°E) to Dornoch (57.89°N, -4.08°E), in Scotland, spanning 220km (Figure 2.1A, Table 2.1). I aimed to spread sites evenly along the transect (mean distance between neighbouring sites = 6.0 km, min = 0.2 km, max = 13.9 km) and they varied in both elevation (Figure 2.1B, Table 2.1) and the type of deciduous woodland habitat. At each site six Schwegler 1B 26mm entrance diameter bird nestboxes were erected at approximately 40m intervals in any configuration. All deciduous-dominated woodlands large enough to accommodate six

nestboxes were considered. The sole exception to this is the highest site (DNS), where there was only sufficient woodland area for four nestboxes, as this is the only available option at this elevation and point of the transect. All sites are outside urban settlements. I used small hole nestboxes to favour use by blue tits and exclude common non-focal species such as great tits and erected them at c.1.5m from the floor with the hole facing away from the prevailing wind. The location of each nestbox was determined using a handheld GPS (Garmin eTrex High Sensitivity) and elevation was obtained (meters above sea level (m.a.s.l)) via the Google Maps elevation API. The elevation of the lowest field site was only slightly above sea level and the highest field site was around the suitable deciduous woodland treeline in Scotland (Pears 1967) (Figure 2.1B, Table 2.1).

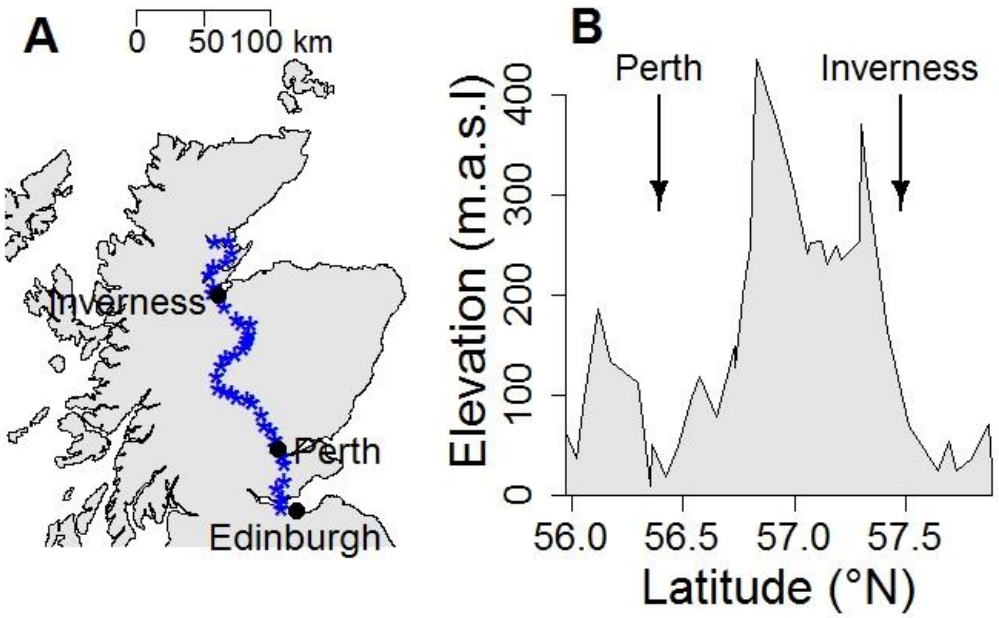


Figure 2.1 A. Map of Scotland showing the locations of all 40 field sites (blue stars), scale, and selected cities as location indicators. **B**. A latitudinal elevation profile of the transect sites, again with selected towns and cities as location indicators.

Table 2.1 Field site details including location and elevation, when the nestboxes were installed, and the years in which each site was intensively studied. Dominant tree defined as the commonest deciduous tree by foliage score, but see Figure 2.2 for more detailed habitat information. Presented on the following page.

Code	Name	Mean Latitude (°N)	Mean Longitude (°E)	Mean Elevation (m.a.s.l)	Nestboxes	Installation Date	2014	2015	2016	Dominant Tree (%)
EDI	Edinburgh	55.98	- 3.40	54	6	04/02/2015		✓	✓	Sycamore (70)
RSY	Rosyth	56.02	- 3.41	37	6	20/01/2015		✓	✓	Sycamore (49)
FOF	Fordell Firs	56.06	- 3.38	87	6	09/12/2013	✓	✓	✓	Sycamore (39)
BAD	Blairadam	56.12	- 3.45	170	6	29/11/2013	✓			Beech (35)
LVN	Loch Leven	56.17	- 3.36	123	6	09/12/2013	✓	✓	✓	Birch (66)
GLF	Glenfarg	56.30	- 3.36	100	6	10/01/2014	✓	✓	✓	Beech (32)
SER	Strathearn	56.35	- 3.40	10	6	20/02/2015		✓	✓	Sycamore (45)
MCH	Moncrieffe Hill	56.36	- 3.38	48	6	29/11/2013	✓		✓	Sycamore (42)
PTH	Perth	56.42	- 3.47	24	6	29/11/2013	✓	✓		Ash (49)
STY	Stanley	56.48	- 3.47	51	6	29/11/2013	✓	✓	✓	Sycamore (30)
BIR	Birnam	56.54	- 3.53	87	6	10/01/2014	✓		✓	Oak (31)
DUN	Dunkeld	56.57	- 3.62	112	6	29/11/2013	✓	✓		Birch (25)
BLG	Ballinluig	56.65	- 3.66	79	6	29/11/2013	✓	✓	✓	Sycamore (46)
KCK	Killiecrankie I	56.73	- 3.77	117	6	09/12/2013	✓	✓	✓	Beech (51)
KCZ	Killiecrankie II	56.73	- 3.78	155	6	20/01/2015		✓	✓	Oak (78)
BLA	Blair Atholl	56.76	- 3.85	175	6	09/12/2013	✓	✓	✓	Beech (38)
CAL	Calvine	56.77	- 3.97	195	6	29/11/2013	✓	✓	✓	Birch (58)
DNM	Dalnamein	56.80	- 4.03	248	6	29/11/2013	✓	✓	✓	Birch (46)
DNC	Dalnacardoch	56.82	- 4.13	363	6	10/01/2014	✓	✓	✓	Willow (42)
DNS	Dalnaspidal	56.83	- 4.22	433	4	19/02/2015		✓	✓	Willow (38)
DLW	Dalwhinnie	56.92	- 4.24	377	6	13/12/2013	✓	✓	✓	Willow (71)
CRU	Crubenmore	56.99	- 4.18	298	6	13/12/2013	✓	✓	✓	Birch (87)
NEW	Newtonmore	57.05	- 4.13	236	6	13/12/2013	✓	✓	✓	Birch (87)
INS	Insh	57.07	- 4.00	248	6	13/12/2013	✓	✓	✓	Birch (68)
FSH	Feshiebridge	57.12	- 3.90	242	6	13/12/2013	✓	✓	✓	Birch (88)
RTH	Rothiemurchus	57.15	- 3.85	228	6	19/01/2015		✓	✓	Oak (87)
AVI	Aviemore	57.19	- 3.84	209	6	13/12/2013	✓	✓	✓	Birch (100)
AVN	Avielochan	57.21	- 3.82	217	6	20/01/2015		✓	✓	Oak (78)
CAR	Carrbridge	57.29	- 3.79	252	6	14/12/2013	✓	✓	✓	Birch (55)
SLS	Slochd Summit	57.30	- 3.92	375	6	19/01/2015		✓	✓	Birch (94)
TOM	Tomatin	57.33	- 3.98	315	6	13/12/2013	✓	✓	✓	Birch (100)
DAV	Daviot	57.41	- 4.15	152	6	14/12/2013	✓	✓	✓	Alder (79)
ART	Artafallie	57.51	- 4.31	60	6	13/10/2015			✓	Oak (73)
MUN	Munlochy	57.55	- 4.28	54	6	14/12/2013	✓	✓	✓	Oak (23)
FOU	Foulis Estate	57.64	- 4.35	17	6	14/12/2013	✓	✓	✓	Sycamore (49)
ALN	Alness	57.69	- 4.29	35	6	14/12/2013	✓	✓	✓	Birch (86)
DEL	Delny Muir	57.72	- 4.13	18	6	14/12/2013	✓	✓	✓	Elm (21)
TAI	Tain Pottery	57.80	- 4.04	23	6	14/12/2013	✓		✓	Birch (32)
SPD	Spinningdale	57.87	- 4.26	71	6	19/01/2015		✓	✓	Oak (86)
DOR	Dornoch	57.89	- 4.08	28	6	14/12/2013	✓	✓	✓	Alder (55)

The study was carried out during the springs of 2014-16, with different sites studied intensively in different years (Table 2.1) and intensive study of 24 sites across all three years of the study, 14 sites across two years and two sites for a single year. Intensively studied field sites were visited every other day throughout the field season (mid-March to late-June) and alternate sites were monitored on each day where possible to avoid artificial autocorrelation as much as possible. Sites with installed nestboxes that were not intensively studied in 2015 and 2016 (those un-ticked in these years in Table 2.1) were omitted from intensive study due to access complications but were visited at least four times during the field season to collect data on blue tit occupancy, clutch size and fledging success. All dates used in this study, unless explicitly indicated otherwise, are ordinal dates counted from January 1st, meaning that April 1st is day 91 in most years and 92 in a leap year.

2.3.2 Habitat

Habitat was recorded around each nestbox at 39 field sites in June-July 2015 and one site in June 2016. I sampled the woodland habitat within a 15m radius of each nestbox. This distance was selected because it was found to provide a fair representation of surrounding habitat and avoided cases of the same trees contributing to the habitat of different nestboxes. To capture variation in tree maturity I assigned every tree with part of its trunk within the 15m radius of the nestbox and a trunk over 40cm in diameter at breast height (approximately 150cm from the ground) to one of three size categories: small (40-99cm girth at breast height (gbh)), medium (100-249cm gbh) and large (>250cm gbh). All measurements of tree size were taken at breast height, so if a tree split below this measure the size of each separate trunk was recorded.

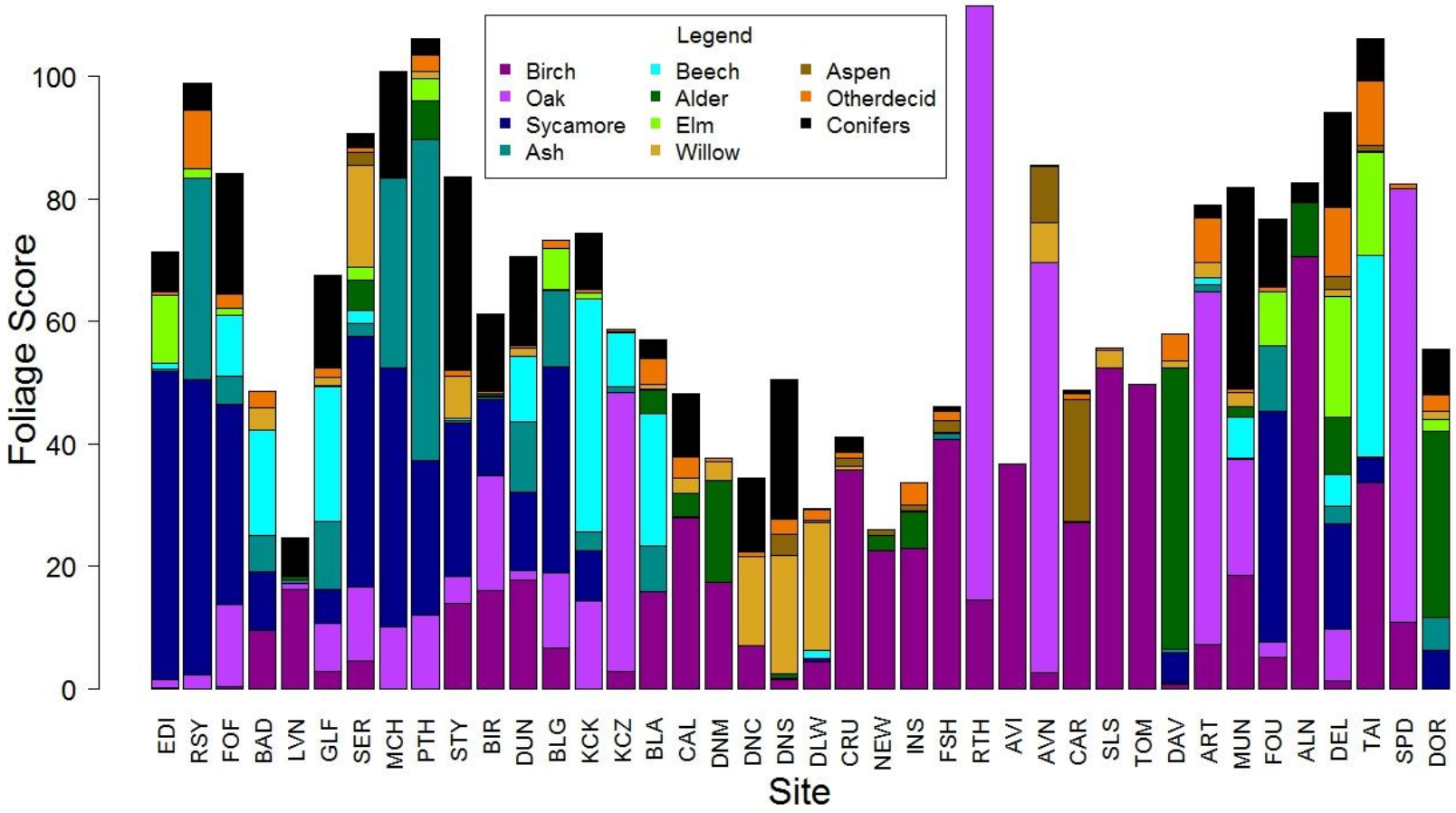
At some sites there were few trunks that qualified under the above definition of a tree, but there were stands of shrub cover (e.g. Hazel *Corylus avellana* and Willow *Salix sp.*) that provided feeding habitat. To accommodate this, three 'stand' classes were constructed. (1) Stand6-20: where 6-20 separate branches emanated from within 20cm of the base of the shrub stand; (2) stand21+: where >20 branches split; (3) When the shrub stand was too impenetrable to count the stems for a stand score, I measured the length and width of the thicket to create a rectangle full of thicket, and estimated the maximum height of the thicket. While converting these stand scores to the foliage provided by a number of trees will only be very approximate, based on visual inspection the following equivalences were used: stand6-20 = 0.5 small trees, stand21+ = 1 small tree and thicket volume x1/30 = n small trees.

Each tree or shrub was identified to genus level and then assigned to focal taxon categories (Table 2.2). Tree identification was to genus level due to substantial evidence of intra-genus hybridisation (e.g. *Betula pubescens x pendula*, *Quercus robur x patraea*, *Salix caprea x cinerea*) along the transect and similar intra-genus ecological properties and associated invertebrate communities (Kennedy & Southwood 1984; Southwood *et al.* 2004). I weighted large, medium and small trees of each genus by the minimum diameter (e.g., $\pi[250/(2\pi)]^2$ for large trees) to obtain an approximate 'foliage score' for each tree genus at each nestbox (see Figure 2.2 for site means). The intention here was to partially represent the ability of larger trees to afford a greater habitat resource and foraging space for blue tits than smaller trees.

Table 2.2 Focal tree taxon categories, detailing the most prevalent tree species along the transect within each category, ordered by mean category foliage score per nestbox (Birch to Aspen) followed by the multi-genera categories (Other Deciduous and Conifers). Categories are at the genus level, or above this level if the taxon is uncommon on the transect (mean genus foliage score per nestbox < 1). Total n = 5921.

Category	Species	n	Size (%)			
			Small	Medium	Large	Stand
Birch	Downy Birch (*Betula pubescens*) Silver Birch (*Betula pendula*)	1929	81	18		1
Oak	Pedunculate Oak (*Quercus robur*) Sessile Oak (*Quercus patraea*)	499	30	66	4	
Sycamore	Sycamore Maple (*Acer pseudoplatanus*)	858	67	32	1	
Ash	European Ash (*Fraxinus excelsior*)	486	73	26	1	
Beech	European Beech (*Fagus sylvatica*)	194	65	27	8	
Alder	Common Alder (*Alnus glutinosa*)	491	85	14		1
Willow	Goat Willow (*Salix caprea*) Grey Willow (*Salix cinerea*) Eared Willow (*Salix aurita*) White Willow (*Salix alba*) Crack Willow (*Salix fragilis*)	481	70	6		24
Elm	Wych Elm (*Ulmus glabra*)	158	73	26	1	
Aspen	Eurasian Aspen (*Populus tremula*)	100	71	29		
Other Deciduous	Common Hazel (*Corylus avellana*) European Rowan (*Sorbus aucuparia*) Hawthorn (*Crataegus monogyna*) Wild Cherry (*Prunus avium*) Bird Cherry (*Prunus padus*) Sweet Chestnut (*Castanea sativa*) Small-leaved Lime (*Tilia cordata*)	330	70	11		19
Conifers	Scots Pine (*Pinus sylvestris*) Common Yew (*Taxus baccata*) European Larch (*Larix decidua*) Norway Spruce (*Picea abies*) Sitka Spruce (*Picea sitchensis*)	395	55	43	2	

Figure 2.2 Bar plot of mean foliage scores per site for each focal taxon category (Table 2.2), with 'Otherdecid' referring to other deciduous trees. Site names from left to right correspond to south to north (Table 2.1).



I characterised variation in woodland habitat based on five measures of the amount of foliage (total, birch, oak, sycamore, willow) and one measure of tree diversity. Foliage scores were calculated at the site level as the mean of the nestbox scores. The motivation for focussing on these four tree species is that birch, oak and sycamore were the three most common focal tree taxa by foliage score along the transect (Table 2.2), and, along with willow, constitute the dominant species at the majority of sites (Figure 2.2, Table 2.1). Total foliage provides a metric for the total foraging resource available to blue tits and is in effect the product of woodland density and maturity, accounting for increases in trees in general of species not included in models individually. Tree diversity was quantified as Simpson's diversity index at the site level across all genera (i.e. 'other deciduous' and 'conifers' categories were split into their constituent genera (Table 2.2)) via the R package 'vegan' (Oksanen *et al.* 2012). This variable was included as greater tree diversity may be correlated with greater prey diversity and abundance (Southwood *et al.* 1982; Fuentes-Montemayor *et al.* 2012) and/or increase the temporal spread of prey availability (Kennedy & Southwood 1984). Across sites the pairwise correlations among habitat variables was < 0.52, implying that co-linearity should not present a problem in the analyses.

2.3.3 Invertebrates

To monitor (mostly flying) invertebrates I installed 2 x 245 x 100mm double-sided yellow sticky traps at c.1.75m above the ground on two randomly selected trees at each intensively studied site (Table 2.1), with the same trees, and when possible branches, used each year. Each sticky trap had a protective cage constructed from 25 x 12mm wire mesh that slotted over it to prevent bird and bat mortalities. Every four days each sticky trap was collected and replaced. Sticky trap use was for the period 22/23 March – 14/15 June 2014, 24/25 March – 16/17 June 2015 and 28/29 March – 16/17 June 2016. I counted all invertebrates over 3mm in length (n=98772) collected by the traps (both sides) and assigned each to at least order level in the following taxa: *Arachnida: Araneae*, *Arachnida: Opiliones*, *Arachnida: Acari*, *Diplopoda*, *Insecta: Ephemeroptera*, *Insecta: Plecoptera*, *Insecta: Psocoptera*, *Insecta: Hemiptera: Heteroptera*, *Insecta: Hemiptera: Auchenorrhyncha*, *Insecta: Hemiptera: Sternorryncha*, *Insecta: Neuroptera*, *Insecta: Coleoptera*, *Insecta: Diptera: Nematocera*, *Insecta: Diptera: Brachycera*, *Insecta: Lepidoptera*, *Insecta: Hymenoptera* and other/unidentified. To quantify repeatability 58 sticky traps were randomly sampled and counted for a second time (26 from 2014, 16 each from 2015 and 2016). Repeatability of total invertebrates on a given sticky trap was then estimated using a

generalised linear mixed model (GLMM) (Bates *et al.* 2015) with Poisson error structure containing year as a fixed effect and site, date, sticky trap ID, sticky trap ID date and residual error as random effects. Regardless of whether repeatability on the latent scale was estimated at the site and date level (i.e. sticky trap ID in the numerator) or transect level (i.e. site, date and sticky trap ID in the numerator), repeatability of total invertebrates was estimated to be > 99%. I subdivided this invertebrate dataset into two roughly equal time periods to partially take into account the major phenological changes in invertebrate abundance over the course of spring. The early time period contained all sticky traps collected from 26th March – 4th May, whilst the late time period constituted those collected from 5th May – 17th June in each year. Site level predictions (ln-scale) for total invertebrate availability in early spring and late spring were estimated using Poisson GLMM's in the MCMCglmm R package (Hadfield 2010) that included site as a fixed effect and sticky trap ID, year and sampling date as random effects.

2.3.4 Birds

At all intensively studied sites (Table 2.1), nestboxes were checked every other day prior to egg-laying. A nestbox was considered occupied if there was at least one egg laid in a lined nest. Clutch size was counted post-incubation initiation and prior to hatching. All nestlings were individually ringed under license from the British Trust for Ornithology and nests were revisited after chicks were 20 days old to ascertain the fledging success/failure of individual nestlings. There was evidence of one second brood in 2014 and this was discounted from all analyses. In 2014 at each of the 30 sites studied in that year (see Table 2.1) 10 waxworms (*Galleria mellonella*) were provided every two days in a plastic cup attached to the same tree as two of the nestboxes until the first egg had been laid. The aim of this supplementary feeding experiment was to understand the role that food availability plays in breeding phenology. However, subsequent analysis revealed that the treatment had no effect on first egg date.

2.3.5 Statistical analyses

All analyses were conducted in R version 3.1.1 (R Core Team 2014). I used spatial GLMMs to study the effects of habitat, biogeography and invertebrate availability on blue tit occupancy (proportion of nestboxes at a site that were occupied by blue tits), clutch size and fledging success (proportion of a clutch that fledged). The motivation for focussing on clutch

size and fledging success (rather than total fledglings) is that it allows the examination of the effects of drivers on these two largely independent components of productivity (with total fledglings the product of the two). However, I also considered a model with total fledglings, presented in the supplementary material. Spatial GLMM's were constructed via the spaMM R package (Rousset & Ferdy 2014) which treats spatial correlation among sites as random effects and I assumed that spatial autocorrelation among sites declines exponentially with distance by fixing nu at 0.5. Occupancy and fledging success were modelled with binomial family errors, and clutch size and total fledglings were log-transformed and modelled with Gaussian family errors. I excluded from analyses nestboxes occupied by coal tits (*Periparus ater*, one in each of 2015 and 2016) and stolen or unavailable nestboxes (two in 2015, one in 2016). Models included habitat variables, latitude, elevation and year as fixed effects. It was possible to include latitude and spatial autocorrelation in the same model as the former describes a linear trend, whereas the latter allows for the correlation to decay with distance over an estimated range in two dimensions. I also included site level predictions of early season total invertebrates in the occupancy and clutch size models and late season total invertebrates in the fledging success and total fledglings models. Nestbox ID was included as a random term in all models.

Nestbox provision can result in blue tit breeding densities that are double natural levels (Dhondt, Kempenaers & Adriaensen 1992) and blue tits preferentially select territories with few neighbours (Serrano-Davies, Barrientos & Sanz 2017). For the occupancy model I tested whether nestboxes led to an increase in blue tit density, by including a two-level factor distinguishing first versus subsequent seasons. Breeding density has been shown to reduce clutch size and fledging success in tit populations across different habitats (Both 1998; Wilkin *et al.* 2006; Dhondt 2010; Sæther *et al.* 2016) and to accommodate such an effect I included blue tit density as the proportion of operational nestboxes occupied at a site in the clutch size, fledging success and total fledglings models.

In all of the above models, site means were used for all predictor variables and all numeric predictor variables were mean-centred for ease of interpretation (Schielzeth 2010). Latitude values were expressed as northing values in units of metres. Maximum likelihood was used for GLMM optimisation. The modelling approach was to construct a full model including all terms, which I did not then seek to simplify. No interactions were included as there were no strong *a priori* reasons for including them. To test the significance of specific individual terms where $t > 1.5$ I used term deletion and likelihood ratio tests to obtain P values. As the

models include multiple terms there is a high probability that some terms will be significant even if the null hypothesis were true. Whilst I don't correct for this, I suggest that this should be borne in mind when interpreting the results. To ascertain whether habitat in general had a significant effect I deleted all habitat terms as a group predictor and compared models with a likelihood ratio test to the full model, with the degrees of freedom equal to the difference in number of estimated parameters.

To evaluate the importance of spatial autocorrelation in each model, I fixed rho = 10000 to simulate negligible autocorrelation and then compared with a likelihood ratio test to the full model. To test the sensitivity of the results to the use of habitat stand scores, these data were excluded and models were re-run and parameter estimates compared. Finally, to contextualise the amount of spatial variance explained by (i) all habitat variables, (ii) the two biogeographic variables and (iii) invertebrate resource availability, each of these predictor blocks were independently removed from the full model and the spatial variance compared with both the full model and a null model that contained only year as a fixed effect and the random and spatial autocorrelation terms.

2.4 Results

Total foliage, oak, sycamore and tree diversity all appear to decrease at higher elevations, with birch and willow displaying the opposite trend (Figure A1). Whereas, birch increases with latitude but the other habitat variables exhibited no clear trend (Figure A1).

The total number of invertebrates sampled on sticky traps varied substantially among sites and dates (Figure A2). Across sites there is little evidence for any latitudinal trend in the amount of invertebrates, whereas there is a decrease in invertebrate abundance with elevation in the early time period (Figure A3B), with the opposite pattern in the late time period (Figure A3D).

Occupancy was not significantly predicted by habitat in general, or by any individual habitat variable (Table 2.3A). Instead there was support for biogeographic variables, with occupancy decreasing with latitude, such that holding other predictors constant (for the year 2014 and with all other variables at their means – the same approach is taken with all other predictions reported below), 70% of nestboxes were predicted to be occupied in the far south of the transect declining to 33% in the far north (Figure 2.3A). Elevation was also a

significant predictor of occupancy, and the probability of occupancy decreased from 79% at sea level to just 13% at the highest elevation (Figure 2.3B). The environmental availability of invertebrates early in the spring, whether the nestbox was in its first available year or a subsequent year, and year, were all non-significant predictors.

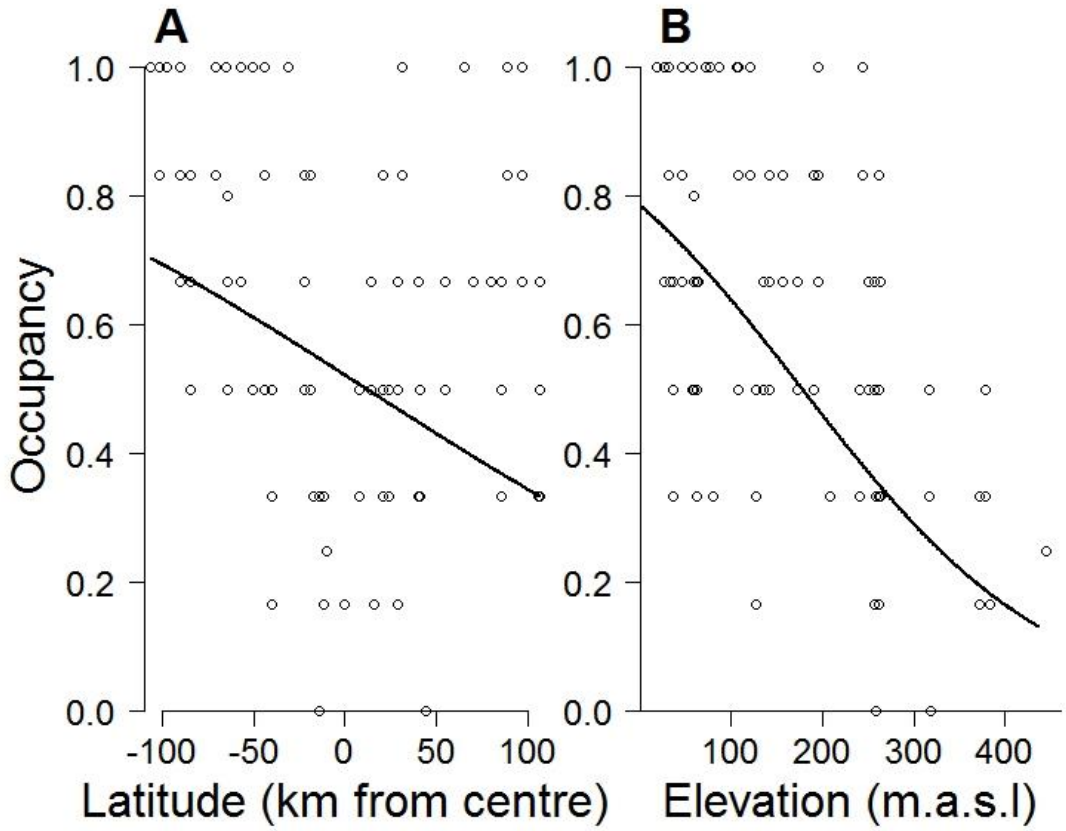


Figure 2.3 The effect of **A** latitude and **B** elevation on nestbox occupancy in blue tits, with all other variables at their mean, in 2014 and in the first spring since site installation.

The mean clutch size was just over eight and varied within years (2014: 8.63 ± 2.07 (mean ± sd), 2015: 7.62 ± 1.82, 2016: 8.08 ± 1.49, total range: 2-14). Habitat was not a significant predictor of clutch size in general (Table 2.3B). Willow was the only significant habitat predictor, such that clutch size was predicted to increase from 8.3 with no willow present to 10.4 with the highest amount of willow found on the transect. There was no significant biogeographic trend in clutch size across latitudes or elevations and no effect of invertebrate availability early in the year, or of blue tit density. Differences in clutch sizes among years were pronounced, with clutch sizes highest in 2014 and predicted to be 12% and 6% lower in 2015 and 2016, respectively.

Table 2.3 Effects on blue tit occupancy, clutch size and fledging success along the transect. Slopes (coefficient) are shown with their associated standard errors (se) from the respective full GLMM's. All significant slopes from fixed effects are presented in bold ($p \leq 0.05$ * ≤ 0.01 ** ≤ 0.001 ***) with individual term p values obtained via term deletion and the habitat group p values (denoted in each column by the bracket wrapping all deleted terms) obtained via group deletion. No significance asterisk implies that predictor or predictor group is not significant. Intercept year is 2014.

	A. Occupancy	B. Clutch Size	C. Fledging success
Fixed Term	**coefficient ± se**	**coefficient ± se**	**coefficient ± se**
Intercept	0.090 ± 0.228	2.14 ± 0.03	1.78 ± 0.16
Total Foliage	0.0054 ± 0.0159	0.00069 ± 0.00108	-0.00027 ± 0.01059
Birch	-0.0039 ± 0.0166	-0.00065 ± 0.00123	**0.025 ± 0.011 ***
Oak	0.0029 ± 0.0145	-0.00041 ± 0.00105	**0.041 ± 0.010 *****
Sycamore	0.013 ± 0.024	0.00092 ± 0.00155	**0.044 ± 0.016 ****
Willow	0.0096 ± 0.0454	**0.011 ± 0.003 ****	-0.056 ± 0.030
Tree Diversity	0.051 ± 0.218	-0.024 ± 0.015	**0.49 ± 0.15 ****
			***
Latitude	**$-7.3\times10^{-6} \pm 3.6\times10^{-6}$ ***	$-3.9\times10^{-7} \pm 2.5\times10^{-7}$	$2.7\times10^{-6} \pm 2.7\times10^{-6}$
Elevation	**-0.0073 ± 0.0029 ***	$-6.6\times10^{-6} \pm 2.4\times10^{-4}$	**0.0061 ± 0.0021 ****
Early Invertebrates	-0.25 ± 0.36	-0.020 ± 0.024	-
Late Invertebrates	-	-	**1.50 ± 0.37 *****
Subsequent Year	0.12 ± 0.50	-	-
Blue Tit Density	-	-0.056 ± 0.068	-0.25 ± 0.44
Year 2015	0.86 ± 0.51	**-0.13 ± 0.03 *****	**-1.84 ± 0.16 *****
Year 2016	0.43 ± 0.59	**-0.066 ± 0.033 *****	**-0.80 ± 0.14 *****
Random Term	**variance**	**variance**	**variance**
Space	0.6	6.5×10^{-9}	1.4×10^{-9}
Nestbox ID	0.2	2.1×10^{-4}	2.0
Spatial Autocorrelation	**parameter**	**parameter**	**parameter**
nu	0.5	0.5	0.5
rho	0.0024	0.0038	5.5×10^{-6}

Spatial variances when predictor blocks were removed: Occupancy: - habitat 0.66, - biogeography 0.86, - invertebrates 0.64, null 1.98. Clutch Size: - habitat 0.0011, – biogeography 7x10-9, - invertebrates 6x10-9, null 0.0032. Fledging Success: - habitat 0.39, - biogeography 0.13, - invertebrates 0.20, null 0.48.

Fledging success, unlike occupancy and clutch size, was predicted by several habitat variables (Table 2.3C, Figure 2.4). Amongst the individual habitat variables, birch, oak, sycamore and increasing tree diversity all predicted a significant increase in the proportion of eggs that survived to fledging. Where oak foliage was at the highest levels found on the transect it predicted fledging rates of 100%, whilst zero oak predicted 80%. The equivalent figures for sycamore and birch were very similar at 97%, 80%, 96% and 79% respectively. Fledging success also increased with tree diversity, with predicted success of 97% at the highest levels of tree diversity on the transect, versus 71% at the lowest. Of the six habitat variables considered, the coefficients for five of these switched sign between the fledgling success and clutch size models. Providing further evidence that site level habitat indices are key predictors of fledging success, when all habitat variables were removed from the full model the spatial variance increased considerably and much more than when biogeographic variables or food availability were removed (Table 2.3 footnotes). These effects of habitat on fledging success are not dominated by year effects, being in the same direction each year (Table A1A-C). In addition to habitat, the availability of late spring flying invertebrates also predicted increased fledging success (from 62% to 97%). Fledging success also increased significantly with increasing elevation, with predictions ranging from 68% to 97% from the lowest to highest elevations, though the latitudinal trend was very shallow and non-significant. Year had a substantial effect on fledging success, with predicted fledging success of 86%, 49% and 73% in 2014, 2015 and 2016, respectively. There was no evidence that blue tit density had any effect on fledging success within the parameters of this study. Quantitatively, the results for the total number of fledglings were congruent to those described here for fledging success, with all coefficients in the same direction and of comparable significance (Table A1D).

Spatial autocorrelation was very weak for both occupancy and clutch size, where the correlation declined to 0.1 by just 959m and 606m respectively, considerably less than the mean distance between adjacent sites along the transect. In comparison spatial autocorrelation was much stronger for fledging success (range at which correlation declined to 0.1 = 200km), which implies that fledging success at even distant sites is correlated. However, a likelihood ratio test comparing these models to a model with very weak spatial autocorrelation was non-significant for all three models ($p > 0.8$ in all models), which suggests that spatial autocorrelation is either weak or the data lacks the power to estimate it well. Of the predictor variable ‘blocks’, spatial variance was best explained by biogeography for occupancy and habitat for clutch size and fledging success (Table 2.3 footnotes).

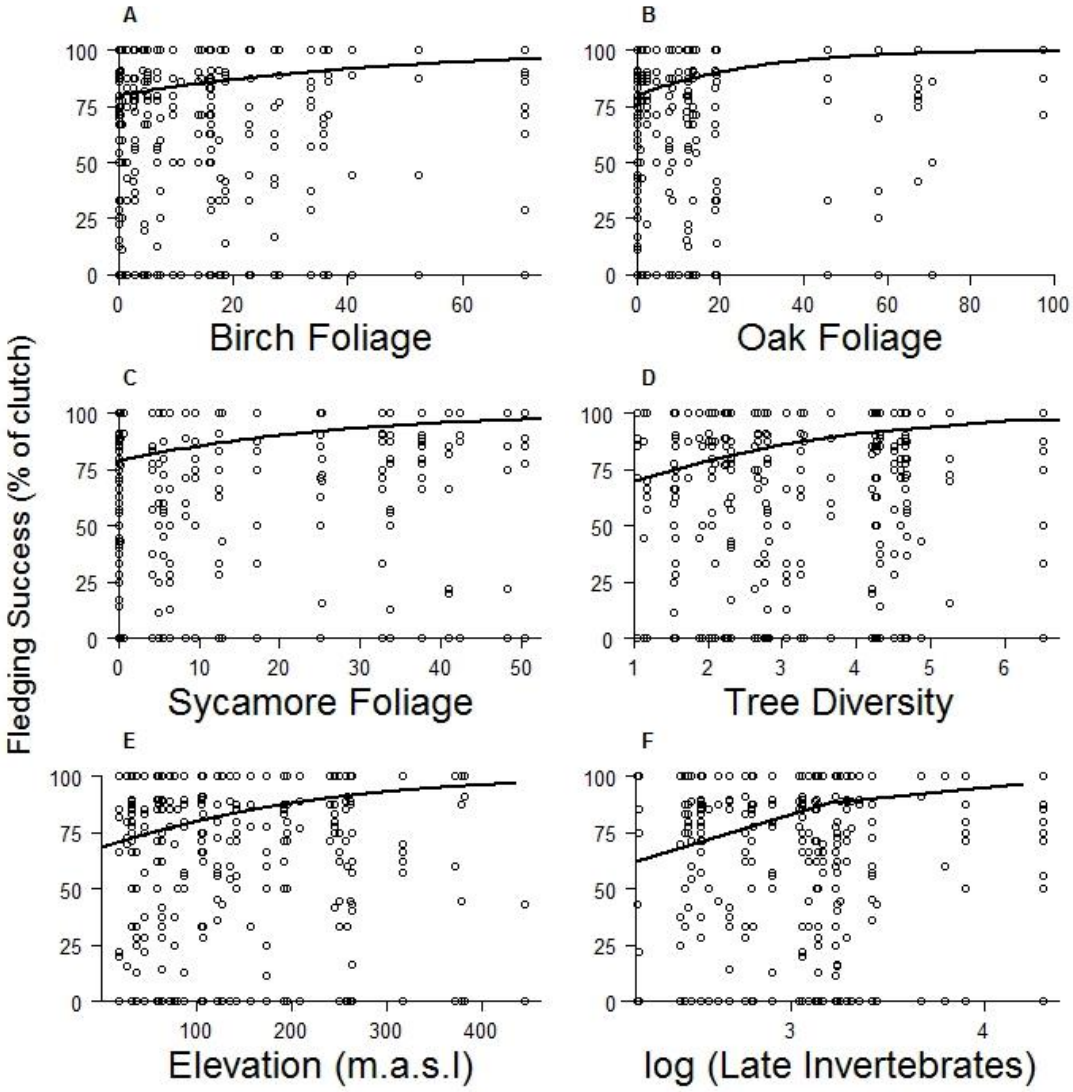


Figure 2.4 Predictors of fledging success: **A** Birch Foliage **B** Oak Foliage **C** Sycamore Foliage **D** Tree Diversity **E** Elevation **F** Late-spring Invertebrate Abundance (log scale). Lines show the prediction, with all other variables at their mean and in 2014.

2.5 Discussion

This study shows that habitat plays a critical role in predicting the fledging success of blue tits, with increasing availability of birch, oak and sycamore and higher tree diversity all having a positive effect. That these patterns are consistent across years provides substantial evidence in support of a genuine general effect in Scotland (Table 2.3, Table A1). In

contrast, habitat did not predict occupancy or clutch size. I propose that this discrepancy between the habitat predictors of early-season breeding decisions and late-season breeding outcomes could suggest that blue tits may not be accurately assessing, or accounting for, the future quality of their breeding habitat when occupying territories and laying clutches. Occupancy is better predicted by biogeography, and declines as elevation and latitude increase, whereas inter-annual variation, probably in the form of untested environmental factors (e.g. rainfall, temperature), is the strongest predictor of clutch size.

Blue tit fledging success was highly sensitive to habitat variables, with the site-level availability of birch, oak and sycamore all positive predictors. These findings broadly agree with earlier work that reports that whilst blue tits are woodland generalists, productivity is highest when certain species are present, particularly oak (Wilkin *et al.* 2009; Amininasab *et al.* 2016). However, whilst previous work has concentrated on differences between major woodland types, such as deciduous versus coniferous (Gibb & Betts 1963; Van Balen 1973) or sclerophyllous (Blondel *et al.* 1993; Lambrechts *et al.* 2004), this study demonstrates more nuanced effects of different constituent species within deciduous woodland, and over a much larger geographic scale.

Oak has previously been used in studies as a proxy for blue tit habitat quality (Wilkin *et al.* 2007; Bell *et al.* 2014), justified on an assumption that oak trees support higher abundances of winter moth caterpillars, a critical dietary component for rearing nestlings. This study corroborates the use of oak availability as a proxy for habitat quality and provides the most comprehensive results to date that an increase in the availability of oak predicts an increase in fledging success. However, sycamore and birch also predict increased fledging success, and this demonstrates that other species in addition to oak provide high quality blue tit habitat. As total foliage, capturing the effect of an increase in the average tree after accounting for the individually analysed tree species, elicits no significant effects on the birds, it can be surmised that the positive effects of oak, birch and sycamore are due to these species providing exceptionally productive habitat rather than this effect simply being a product of a local increase in trees in general. Biogeographic variables and breeding density did not significantly predict fledging success, the latter differing from some previous studies (Dhondt *et al.* 1992; Wilkin *et al.* 2006). However, the maximum number of nestboxes per site was low (n=6) and I modelled the effect of breeding density as an absolute effect consistent across sites, which does not take into account among site differences in average tit density and may explain why no effect of density is detected.

In contrast to fledging success, the other component of productivity studied, clutch size, was not significantly predicted by habitat, or any individual habitat variables, with the exception of a slight positive effect of willow availability. Given the apparent lack of variation in clutch sizes across habitats this could imply that there is no local adaptation to habitat. One explanation for this would be if adaptation to key habitats in the wider landscape dominates, as earlier work on blue tit clutch sizes has found (Blondel *et al.* 1993; Dias & Blondel 1996) and so clutch size is less sensitive to habitat than fledging success (Arriero *et al.* 2006). I also found that many variables had an opposite directional effect on the predicted slope for clutch size as they did for fledging success; this might be explained by individual females making suboptimal large reproductive investments in early spring in habitats that later prove to be poor. Indeed, it is possible that overinvestment in clutch size actually reduces subsequent fledging success (Lack 1947; Monaghan & Nager 1997). This may suggest that habitats with a high quality resource early in the breeding season differ from those that provide a high quality resource late in the breeding season. One explanation for this phenomenon is tree phenology, whereby early leafing trees and habitats may support higher prey abundances early in the season whilst food peaks tail off later on, with late leafing trees, or trees with full-season growth (Niemela & Haukioja 1982), having the opposite tendency. Such temporal asynchronicity in invertebrate abundances across tree species (Southwood *et al.* 2004; Veen *et al.* 2010) could help explain why increasing tree diversity elevates eventual productivity, providing a suitable environment for the entirety of the breeding season through the diversity of leafing times maintaining a more sustained and reliable temporal availability of prey.

Whilst blue tits did not seem to predict high quality local habitats within a year, clutch size and fledging success varied substantially among years with coincident trends, although the two were correlated based on just three years of data. If genuinely positively correlated, this is consistent with high quality versus low quality years being a major source of variation in reproductive success within this system (Perrins 1979; Tremblay *et al.* 2003). That there was no evidence of a latitudinal gradient in clutch size at this scale agreed with previous studies (Fargallo 2004; Evans *et al.* 2009).

Occupancy, like clutch size, was not significantly predicted by habitat. This may imply that blue tits occupy nestboxes across different habitats at random. However, more likely is that population densities on larger spatial scales determine occupancy. Blue tit populations in the

UK are currently at a high ebb (Balmer *et al.* 2013) and an effect of this may be that even low quality 'sink' habitats become occupied (Bellamy *et al.* 2000). Biogeographic variables did however predict occupancy, with occupancy highest at low elevations and decreasing further north, agreeing with other work (Fargallo 2004). However, the findings of this study suggest that these biogeographic trends occur at a finer latitudinal and elevational scale than previously reported. A decrease in occupancy with latitude and elevation must reflect the impact of environmental variables beyond those captured by site-level habitat metrics, and could include tolerance to temperatures at particular times of year or even the frequency of supplementary feeding (Robb et al 2008), as in the focal area human population density decreases with both latitude and elevation and blue tit density increases between low and moderately high human population densities (Tratalos *et al.* 2007).

To summarise, I find that the availability of oak, birch, sycamore and tree diversity predict increased blue tit fledging success, whereas the effects of habitat on occupancy and clutch size are much weaker, which may imply that blue tits are not able to predict among habitat variation in the future availability of resources. One of the implications of blue tit breeding parameters differing among habitats is that it may not be appropriate to extrapolate insights from the commonly-studied mature (often oak) habitats to others and habitat should be taken into account when predicting demographic changes based on trophic mismatch theory.

Chapter 3

Disentangling the environmental predictors of spatial and temporal variation in blue tit reproductive phenology

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Dalnacardoch

3.1 Abstract

Establishing the cues or constraints involved in timing reproductive phenology is key to predicting future phenological responses to climate change. This study aimed to identify whether aspects of the environment predict the timing of nest initiation and egg laying date in an insectivorous woodland passerine, a popular system for studying phenology. Blue tits (*Cyanistes caeruleus*) are used as a model species and fieldwork conducted along a 220km transect of 40 woodlands across Scotland surveyed 2014-16. I performed both single- and multi- predictor models of two measures of breeding phenology, the initiation of nest building and first egg date, considering day- and night-time temperature, tree phenology, prey abundance and photoperiod as predictors. Whilst all variables were significant predictors of breeding phenology when considered individually, in the multi-predictor model night-time temperature in early spring was the most important predictor of both nest initiation and lay date (slope = -3 days/°C). I suggest that this may provide support for a thermal energetic constraint to phenology, rather than a response to increasing temperatures as a cue. Invertebrate prey abundance also significantly predicted lay date, advancing it by up to nine days, but had no significant effect on nest initiation. Tree phenology, day-time temperature and photoperiod were unimportant in predicting the variation in blue tit reproductive phenology observed in this study in the multi-predictor models. By analysing all plausible factors in a single model from a natural setting for the first time, this refines our understanding of the principal factors influencing the timing of blue tit reproductive phenology and advances our knowledge of how temperature operates as a predictor.

3.2 Introduction

Global climate change is seeing a rise in ambient air temperatures and causing the advance of spring phenological events (Walther *et al.* 2002; Thackeray *et al.* 2016) in the northern hemisphere by an average of 2.3 days per decade (Parmesan & Yohe 2003). The timing of phenological events is often critical to the organisms involved, allowing them to develop and/or reproduce under more favourable environmental conditions, which could be purely abiotic, such as temperatures, but often involve temporal interactions with organisms at other trophic levels, such as avoiding predation or coinciding with high prey abundance (Visser *et al.* 1998; Miller-Rushing *et al.* 2010). Individuals that mistime such phenological events may incur considerable fitness costs (Winder & Schindler 2004; Both *et al.* 2004a). However, not all organisms or trophic levels are advancing their phenologies at the same pace in relation to climate change, as they respond to diverse environmental cues or to similar cues dissimilarly (Durant *et al.* 2007; Gienapp *et al.* 2014; Thackeray *et al.* 2016). This can cause trophic mismatch, whereby consumers become deleteriously temporally asynchronous with an important resource (Miller-Rushing *et al.* 2010).

Predicting how mismatch will affect individuals and populations in the future requires detailed knowledge of the aspect(s) of the environment the interacting species use to schedule their phenological events and the magnitude of their responses to these environmental variables (Lyon, Chaine & Winkler 2008). Pinpointing these environmental predictors of phenology has proved challenging in many taxa. Many organisms, including plants, invertebrates and vertebrates vary their phenology from year-to-year correlated with variation in temperature (Visser *et al.* 1998; Roberts *et al.* 2015; Phillimore *et al.* 2016). Some away from the tropics utilise photoperiod as a reliable signal of longer days and therefore an approaching spring (Lofts & Murton 1968; Zohner & Renner 2015), whilst still others are thought to utilise biotic correlates, such as increasing prey abundance or phenological events occurring at lower trophic levels (Bourgault *et al.* 2010; Cole *et al.* 2015). Some of these environmental variables might act as either cues, signalling future conditions and allowing determination of phenological strategies, or constraints, prohibiting advancing phenology until certain abiotic or nutritional conditions are met.

Hole-nesting passerine birds are often used as a model system for studying phenology and mismatch due to the ease of studying breeding phenology (e.g., egg-laying), the importance of phenological events for, and the convenient measurability of their consequences on,

fitness. There has been a particular focus on the oak – caterpillar – insectivorous woodland passerine food-chain (Visser *et al.* 1998; Buse *et al.* 1999; Charmantier *et al.* 2008). In this system, there is an ephemeral superabundance of caterpillars in spring, feeding on young leaves before the trees add tannins as a defence to herbivores (Feeny 1970). Synchronising reproduction with this peak increases the number and quality of successful fledglings for the birds (Wilkin *et al.* 2009; Burger *et al.* 2012). Despite the popularity of this system, the environmental variables the passerine birds use to initiate their reproductive phenology remain unclear (Caro *et al.* 2013). Although there is often a genetic basis to breeding phenology (Husby *et al.* 2010; Gienapp, van Noordwijk & Visser 2013), there has been little evidence thus far of an evolutionary response, with responses mainly involving plasticity (Charmantier & Gienapp 2013), and possibly learning (Grieco, van Noordwijk & Visser 2002; Hušek, Lampe & Slagsvold 2014). The birds must anticipate the timing of the peak in advance, in the absence of perfect information, as they require time to select territories and mates, nest build, lay and incubate eggs, which takes roughly a month (Perrins 1979; Visser *et al.* 1998). The major competing explanations are photoperiod, temperature, tree phenology and prey (invertebrate) abundance (Thomas *et al.* 2010), with mixed support for each and no definitive consensus.

Photoperiod is important in regulating avian reproductive cycles away from the tropics, with increasing daylight hours indicating approaching favourable breeding conditions (Lofts & Murton 1968). Sudden, considerable (17 hour days) and sustained exposure of blue tits to artificial photostimulation in December causes them to breed three months early when supplied with ad-lib food (Lambrechts & Perret 2000). It operates through stimulating gonadal and follicular growth and signalling song production (Dawson *et al.* 2001; Helm *et al.* 2013). Gonadal development can be rapid after the spring equinox (Silverin, Viebke & Westin 1989), and has been stimulated experimentally in great tits via a single artificially long day in winter (te Marvelde, Schaper & Visser 2012), although this treatment did not influence eventual lay date. There is roughly a seven to eight week interval between the onset of rapid gonadal development and egg laying reported in wild tits, but this was reduced to five weeks under artificial photostimulation (Silverin *et al.* 1989; Lambrechts & Perret 2000). This plasticity indicates that photostimulation is necessary to initiate reproduction but not in itself sufficient and beyond photostimulation other supplemental indicators fine-tune timing (Caro *et al.* 2007). This hypothesis is supported by the observation that male gonads within Corsican blue tit populations have been found to develop synchronously despite widely different eventual laying dates (Caro *et al.* 2006). It has also been surmised that a

photoperiodic predictor may be of greater import at high latitudes where supplemental cues may arrive too late to be of informative value (Silverin *et al.* 2008). Distinct populations can display locally adapted photoperiodic responses (Lambrechts *et al.* 1997b; Liedvogel *et al.* 2009; Perfito *et al.* 2012), providing one explanation as to how and why phenology is divergent between populations at the same latitude, and therefore photoperiod, within a year, but as photoperiod is inter-annually consistent it cannot be responsible for substantial differences in phenology in the same individual or population between years (for instance in 95% of years the annual mean blue tit and great tit lay dates can range as much as +/- 10 and 12 days of the multi-year mean, respectively (Phillimore *et al.* 2016)).

The average spring temperature during a sensitivity window is known to be a strong correlate of clutch initiation in woodland passerines (Wesolowski 1998; Charmantier *et al.* 2008; Visser *et al.* 2009). For tit species a rise of 1°C usually elicits a 3.5-4.5 day advancement in clutch initiation (Visser *et al.* 1998; Phillimore *et al.* 2016) but the means by which average temperature affects the birds is unknown (Caro *et al.* 2013). Some experimental studies provide evidence that the average temperature itself is a cue sensed by the birds (Visser *et al.* 2009), or that increasing temperatures trigger reproduction (Schaper *et al.* 2012). Other studies suggest that temperature may act as a constraint, energetically limiting when the birds are able to begin the costly act of egg production (Stevenson & Bryant 2000), as in the space of a fortnight or less a female blue tit can lay a clutch that is in excess of 130% of her body weight (Perrins 1970; Perrins & Birkhead 1983). In support of the constraint hypothesis, yolk production correlates with laying date (Caro *et al.* 2009) and cooling nestboxes retards egg formation in starlings (*Sturnus vulgaris*) (Meijer *et al.* 1999), and reduces egg volume but does not significantly delay lay date in blue tits (Nager & van Noordwijk 1992). All previous studies have combined day and night temperatures, but it is possible that they may act quite differently, with day-time temperatures signalling rising maxima and offering a cue of advancing conditions, and night-time temperatures signalling minimum temperatures and therefore thermal constraints.

Whether temperature acts directly as a predictor of woodland passerine breeding phenology, or indirectly via a correlated factor, such as tree phenology or invertebrate abundance, is also debated. Tree leafing phenology, most frequently oak or birch, has been reported as a significant positive correlate of lay date over time (Nilsson & Källander 2006; Thomas *et al.* 2010) and across space at the site level (Hinks *et al.* 2015). However, in estimating the effect of tree phenology, some studies fail to include temperature in their models, so the

estimated coefficient may be an artefact of both birds and trees responding to a temperature cue. However, where vegetation types differ substantially, vegetation phenology can be a better predictor of laying date than temperature, as shown in Mediterranean blue tits (Bourgault *et al.* 2010). It has been proposed that consuming the emerging buds would allow the birds to derive chemical cues, such as in the edible dormouse (*Glis glis*) (Pilastro, Tavecchia & Marin 2003), but dietary bud use is minimal and temporally consistent (Bourgault *et al.* 2006) and an experiment that involved inserting leafing branches into aviaries reported no effect on lay date (Schaper *et al.* 2011). Artificial supplementary feeding of passerines, however, has been found to advance lay dates by a few days to a week (Robb *et al.* 2008a; Seward *et al.* 2014), including in woodland insectivores (von Bromssen & Jansson 1980; Nager *et al.* 1997), from which we can infer that food availability can be a predictor of breeding phenology with a limited magnitude. Food availability could predict breeding phenology by increasing available body proteins to form eggs (Schoech & Bowman 2003). Manipulation of resources has been found to elicit greater responses in poorer years (Nager *et al.* 1997) and territories (Svensson & Nilsson 1995), indicating a possible alleviation of environmental nutrient limitation. Nutrient limitation could also be responsible for later lay dates among parasitised individuals (Allander & Bennett 1995). As far as I am aware no previous analysis has tested the role of natural food resource availability as a phenological cue in this system.

The aim of this study is to establish which factors are responsible for spatial and temporal variation in blue tit reproductive phenology, teasing apart the contributions of photoperiod, temperature, tree phenology and invertebrate abundance using a 220km transect of 40 field sites across Scotland. In contrast to traditional single-site approaches to studying woodland bird phenology, this study design allows substantial independent variation between these factors that often co-vary within a single site (Figure 1.2). In addition, I will examine predictors of nest initiation date as well as first egg date (lay date), as different aspects may control the timing of the onset of nest building to the onset of laying. This could allow for fine-tuning adjustment throughout the breeding season (Cresswell & McCleery 2003; Simmonds *et al.* 2017).

3.3 Methods

3.3.1 Study system and transect

This study was conducted along a 220km transect of Scotland incorporating 40 woodland field sites with nestboxes holding breeding blue tits, detailed in 2.3.1 during 2014-2016. All dates used in this study, unless explicitly indicated otherwise, are ordinal dates counted from January 1st, meaning that April 1st is day 91 in most years and day 92 in a leap year.

3.3.2 Temperature recording

Temperature was monitored by two Thermachron iButtons (model DS1922L-F5), which were installed at opposite ends of each site from 15/2/14, 21/2/15 and 23/2/16 until 12/6 every year. They were secured 1.5m high on the north side of a tree to avoid direct sunlight in a waterproof white pot with a 20mm-diameter hole in the bottom to allow ambient air circulation. iButtons were installed at least 24 hours before first recording and temperatures were recorded every hour on the hour to a sensitivity of 0.0625°C. Mean spring temperatures at each site in each year are displayed in Figure B1.

3.3.3 Habitat and tree phenology

Habitat surveys were conducted at all 40 field sites as detailed in section 2.3.2. Tree phenology was studied at each intensively studied site (section 2.3.1) in 2014 by selecting six focal trees, which were the nearest deciduous tree with a trunk diameter ≥ 20cm to each nestbox, and identifying them to genus level. If there was oak or birch present at a site but one had not been selected in the original six trees by the above method, up to six of each species present were identified, numbered and a die rolled to randomly choose one individual of each species present, resulting in 6-8 focal trees per site. In subsequent years (2015-16) these same individual focal trees were used wherever possible (individual consistency 2014-15 = 80%, 2015-16 = 97%), and additional trees were added so that each site had 8-10 focal trees. These extra trees were selected by using the method described above for oak and birch but extending this to sycamore and willow. If there were less than eight focal trees at the site by this point, the random method above was used on randomly selected deciduous trees of species typical of the local habitat to give each site at least eight focal trees broadly representative of the local habitat. Care was also taken to ensure that at least four of the focal trees at each site had a branch low enough to reach for caterpillar

research in 2015-16 (see Chapter 5) by adding extra trees up to a maximum of 10. One site (DNS) only had four nestboxes unlike the other 39 sites, and at this site the two closest applicable trees to each nestbox were selected to give eight focal trees.

On every visit (every other day) until complete leafing, each focal tree was visually inspected using binoculars for ten seconds. The phenology of each focal tree was tracked, recording the dates of: (i) first bud burst (FBB) – when the green leaf first emerges from the earliest bud on any part of the tree, and (ii) first leaf (FLF) – when the first leaf on any part of the tree is fully unfurled and looks to be the correct shape, if not eventual full size, for the leaf of that tree species (Wesolowski & Rowinski 2006b; Hinks *et al.* 2015).

Table 3.1 Detailing the number of focal trees studied of each taxon each year, with the percentage of intensively studied sites (2014 n=30, 2015 n=35, 2016 n=37) with at least one focal tree of this taxon (site coverage), ordered by focal tree number in 2016, followed by site coverage in 2016. Total focal tree n=186 in 2014 (mean 6.2/site), 293 in 2015 (mean 8.4/site) and 313 in 2016 (mean 8.5/site). Species within each tree taxon along the transect are detailed in Table 2.2.

Tree Taxon (*Genus*)	2014		2015		2016	
	Focal Trees	Sites (%)	Focal Trees	Sites (%)	Focal Trees	Sites (%)
Birch (*Betula*)	85	93	118	97	123	97
Oak (*Quercus*)	19	40	48	57	53	57
Sycamore (*Acer*)	29	47	30	37	33	38
Willow (*Salix*)	7	13	20	31	22	32
Alder (*Alnus*)	15	30	22	31	22	30
Beech (*Fagus*)	13	27	17	23	17	22
Ash (*Fraxinus*)	7	20	10	20	11	19
Elm (*Ulmus*)	2	3	7	17	8	19
Rowan (*Sorbus*)	6	17	8	14	8	14
Aspen (*Populus*)	2	3	6	9	7	11
Hazel (*Corylus*)	3	10	5	14	4	11
Cherry (*Prunus*)	0	-	2	3	2	3
Chestnut (*Castanea*)	0	-	0	-	2	3
Lime (*Tilia*)	0	-	0	-	1	3

3.3.4 Invertebrate phenology

Invertebrate phenology was monitored over four day intervals by yellow sticky traps, as discussed in 2.3.3 and total invertebrate numbers calculated as the sum of all constituent taxa. The two randomly selected trees were both drawn from the focal trees described in 3.3.3.

3.3.5 Blue tit phenology

All nestboxes (26mm hole Schwegler 1B) at intensively studied sites were checked every other day throughout the field season. The nest initiation date (hereafter N1) was recorded when either the entire floor of the nestbox was covered with nesting material, or the nesting material had built up to ≥45mm depth at the front of the nestbox (measured from the bottom of the exterior of the nestbox to the top of the nesting material bulk, excluding stray strands). First egg date (FED) was defined as the date at which the first egg was laid in a lined nest, calculated as the previous day if two eggs were found as blue tits lay one egg per day, generally in the early morning (Perrins 1970). One second brood was excluded from all analyses. N1 occurred 19.2 days before FED on average across all years. In 2014 at each of the 30 sites studied in that year (see Table 2.1) 10 waxworms (*Galleria mellonella*) were provided every two days in a plastic cup attached to the same tree as two of the nestboxes until the first egg had been laid. The aim of this supplementary feeding experiment was to understand the role that food availability plays in breeding phenology. However, subsequent analysis revealed that the treatment had no effect on FED.

3.3.6 Statistical analyses

Temperature as a predictor of blue tit reproductive phenology

In order to identify the time period during which average temperature best predicts phenology, I adopted a sliding window approach (Husby *et al.* 2010; Phillimore *et al.* 2016), and considered a wide range of start dates (days 54-100) and durations (10-60 days) to the windows. However, this resulted in a flat likelihood surface as among site variation in temperature is highly correlated across days (this is much less true across years), and left little information from which to infer if temperature in one time period is a better predictor of phenology than another. Therefore, I used two time periods deduced from British blue tit breeding data in Phillimore *et al.* 2016. The first, temp_i, covered the mean temperature for the period of days 75-128 (16th March – 8th May in non-leap years) and was the best predictor time window from blue tit data across the entirety of the UK. The second, temp_latvar, covered the mean temperature for the period of days 76-134 (17th March – 14th May) and was the best predictor time window when latitudes were allowed to vary in their time windows, centred on the mean latitude of the transect for this study. In addition to these 24-hour mean temperatures, mean day-time (07:00 – 18:59hrs) and night-time (19:00 –

06:59hrs) temperatures were also calculated for both of these time periods. These times were chosen as they split the temperature dataset equally between night- and day- time temperatures, avoiding any bias that would be incurred from estimating either from more data points, and are the closest twelve-hour cycles to equate to sunrise and sunset at the beginning of April in the centre of the transect. Mean day- and night- time temperatures were analysed instead of minimum and maximum temperatures as they smooth extreme and/or inaccurate temperature recordings and can be seen as more biologically relevant to thermoregulating organisms, rather than merely capturing a small period of time or extreme event. These six mean temperatures (temp_i_24hr, temp_i_day, temp_i_night, temp_latvar_24hr, temp_latvar_day, temp_latvar_night) were individually considered as a single fixed effect predictor of FED in linear mixed models (LMM) implemented using lme4 (Bates *et al.* 2015), with site and year as random effects and using maximum likelihood. I assume that the effect of temperature on phenology is similar across space and time, as found by Phillimore *et al.* 2016, meaning that I can estimate just a single slope. I used Akaike Information Criteria (AIC) for model comparison (Burnham & Anderson 2004), and also compared all models to a null model which included all random terms but only the intercept as a fixed effect. The model with the lowest AIC was selected as the best temperature predictor of FED. As a follow-up test of the importance of night versus day time temperature I included both fixed terms in a single GLM.

Previous avian studies have primarily focused on FED as a measure of blue tit reproductive phenology and N1 has been overlooked (Tomás 2015). As a consequence there are no published estimates of the time window of maximum thermal sensitivity available for N1. I thus used a sliding window approach due to no viable alternative being available, to find the best temperature predictor of N1, with starting days 54-100 and durations of 10-60 days considered. Despite another flat likelihood surface (Figure B2), the best predicting timeframe was identified and termed temp_sw. Temp_sw (24hr, day and night as detailed above) periods were then used as fixed effects in LMMs as detailed above for FED, with the same random effects and a similar null model as used in the FED model. The model with the lowest AIC was selected as the best temperature predictor of N1, and this was validated by a similar LMM to that described above but containing both day and night mean temperatures for temp_sw.

Tree Phenology as a predictor of blue tit reproductive phenology

I calculated the percentage composition at each site of each tree genus comprising each site without reference to tree size, and calculated the mean FBB and FLF of each tree genus at each site in each year. These were then multiplied together where the FBB (or FLF) was known from a sampled focal tree of that genus in that year at that site and all values at a single site in a single year summed. A site weighted mean FBB (or FLF) was then calculated for each intensively studied site in each year by dividing these values by the total percentage of the habitat at that site that was represented by the focal trees for which I had phenology data at the site, as shown in Equation 3.1 (2014 range in coverage of site habitat by focal trees 31.8-100%, mean 77.7%, 2015 range 60.7-100%, mean 84.3%, 2016 range 65.5-100%, mean 85.4%, with much of the uncovered habitats being coniferous tree species, for which phenology was not tracked).

Equation 3.1 Calculation to obtain weighted site mean budburst at a single site in a single year, where f = frequency of tree at site (percentage), b = mean first budburst of tree species at site per year and 1-14 denote tree taxa. Weighted site mean first leaf was calculated the same way.

$$\frac{\sum_{i=1}^{n=14} f_i \, b_i}{\sum_{i=1}^{n=14} f_i}$$

This local tree-species-insensitive weighted mean FBB (or FLF), where the blue tits are assumed to use all of the available habitat equally as a predictor, was then assigned to its respective site per year and used as a fixed effect to predict N1 and FED in LMM's with site and year as random effects using the MCMCglmm (Hadfield 2010) R package. These were set up as multi-membership models whereby each tree genus in Table 3.1 studied as a focal tree in at least one site in every year had its 'impact' calculated (deviance from mean effect of FBB/FLF on blue tit phenology) over and above that explained by the null weighted mean FBB (or FLF) to deduce if the phenology of certain tree genera were more important than others in predicting blue tit breeding phenology. The posterior distribution of the among-species multi-membership variance was used to assess whether the effect of tree phenology on blue tit phenology varied among tree species. Models were compared on the basis of Deviance Information Criteria (DIC). FLF was not considered as a predictor of N1 as N1 almost always occurs earlier than FLF (mean FLF was approximately 20 days later than

mean N1), so FBB was included as the only biologically plausible tree phenology predictor of N1. Although on average weighted FBB similarly occurs slightly after N1, FBB is the earliest measure of tree phenology recorded and could correlate with, and represent, earlier unrecorded tree phenology stages which the birds may respond to and is therefore still considered as a possible predictor of N1.

Invertebrate abundance as a predictor of blue tit reproductive phenology

Total invertebrate numbers were logged (log x+1) for each sticky trap due to the log normal distribution of abundances and mean totals per site collection day were calculated. The exponential (exp x-1) of these totals was then divided by four and then logged again (log x+1) and this value used as an estimate of the daily log invertebrate abundance across the four collection days. A sliding window approach was then used to find the time period during which average invertebrate availability best predicted N1 and FED (Figures B3 and B4). The mean invertebrate abundance of each possible time period was used as a fixed predictor of N1 and FED in a maximum likelihood LMM in lme4 with site and year as random effects, with starting dates 82-100 and durations of 10-60 days considered. Models were compared on the basis of AIC.

Full Models of Predictors of Blue Tit Reproductive Phenology

The first full model (Full) used to analyse the predictors of blue tit reproductive phenology was a multi-membership LMM in MCMCglmm. N1 and FED were the responses, in separate models, with the best temperature predictor, the best invertebrate phenology predictor, the best tree phenology predictor (all respective for each response) and latitude (as a proxy for photoperiod) included as fixed effects, with site and year as random effects and including all tree genera as multi-members of the best tree phenology predictor (see description of this above). In a second full model (-Multi) I removed the term allowing for multi-membership of each tree genera to determine whether this extra structure was affecting the results. I compared both models against a null (Null) model, with no fixed predictors of each response and site and year as random effects. The last full model used (spaMM) for each response was a LMM allowing for spatial autocorrelation in the spaMM package (Rousset & Ferdy 2014). The response and all fixed and random effects were as in the –Multi model. This model incorporated a latitude and longitude Matern spatial autocorrelation term (using the UK national grid), allowing for an exponential decay (nu fixed at 0.5).

3.4 Results

3.4.1 Temperature as a predictor of blue tit reproductive phenology

The best predictor period for N1 from the sliding window were days 66-92 (7th March – 2nd April in non-leap-years), which was sufficiently early in the year to be a plausible predictor window and I termed this period temp_sw (see methods). All mean temperatures considered returned a significant negative slope with N1 and FED, and all were a significant improvement on their respective null models (Table 3.2).

When predicting FED, temp_i produced lower AIC values, and steeper slopes, than temp_latvar for each subcategory and was thus the better predictor of the two time windows tested (Table 3.2). Across both N1 and FED, mean night-time temperatures were significantly better predictors than mean day-time temperatures (ΔAIC N1 = 4.5, FED = 11.3) or 24hr temperatures (ΔAIC N1 = 1.2, FED = 3.3). The best temperature predictor of N1 found in this study was temp_sw_night and the best temperature predictor of FED was temp_i_night (Table 3.2). Under the best model conditions, N1 was predicted to occur on day 111 when mean nightly temperatures during the temp_sw period were 2°C, advancing to day 99 when mean nightly temperatures during this period were 6°C. FED, on the other hand, was predicted to occur on day 131 when mean nightly temperatures during the temp_i period were 3°C, advancing to day 116 when mean nightly temperatures during this period were 8°C, with all temperature values described above akin to the lowest and highest mean nightly temperatures experienced on the transect during the respective periods in 2014-16 (Figure 3.1).

As a *post-hoc* test of whether night temperatures were indeed better predictors of N1 and FED than day temperatures, both night and day temperatures were included as predictors in the same model, one for each (for the period temp_sw to predict N1 and for the period temp_i to predict FED). Whilst the night temperature slopes stayed fairly constant for both N1 and FED, the day temperature slopes were much reduced and non-significant, consistent with night temperatures being better predictors of the timing of N1 and FED than day temperatures (LMM's: N1 model: intercept 117.0 ± 6.2, mean day temperature slope -0.03 ± 1.44, mean night temperature slope -3.04 ± 1.43, AIC 3135.5. FED model: intercept 138.9 ± 6.2, mean day temperature slope 0.13 ± 0.98, mean night temperature slope -3.09 ± 0.90, AIC 2438.9).

Table 3.2 Temperature predictors of N1 and FED, with slopes (b) and their associated standard errors (se) estimated from LMM's (see methods), together with the AIC value of each for comparison. Temperatures refer to mean temperatures across ordinal dates as follows: Temp_sw = 66-92, Temp_i = 75-128, Temp_latvar = 76-134 (see 3.3.2).

Response	Temperature	Predictor	Intercept ± se	b ± se	AIC
N1	Null		104.5 ± 1.4		3145.6
	Temp_sw	24hr	122.0 ± 4.0	-3.09 ± 0.70	3134.7
		Day	124.6 ± 5.0	-2.76 ± 0.68	3138.0
		Night ‡	**116.9 ± 2.8**	**-3.06 ± 0.67**	**3133.5**
FED	Null		123.3 ± 2.1		2464.5
	Temp_i	24hr	146.8 ± 4.6	-3.23 ± 0.62	2440.2
		Day	142.7 ± 6.4	-2.15 ± 0.69	2448.2
		Night ‡	**139.7 ± 3.3**	**-3.00 ± 0.58**	**2436.9**
	Temp_latvar	24hr	143.6 ± 6.1	-2.66 ± 0.78	2446.3
		Day	134.7 ± 7.7	-1.20 ± 0.78	2452.9
		Night	139.7 ± 4.1	-2.85 ± 0.67	2441.0

‡ These models are the best temperature predictors of N1 and FED respectively and are presented in bold. Random effect variances for **N1** models were: **Null** site = 28.3, year = 3.0, residual = 96.2 **24hr** site = 20.6, year = 0, residual = 96.1 **Day** site = 20.5, year = 0, residual = 96.9 **Night** site = 21.6, year = 0, residual = 95.5. Random effect variances for **FED** models were: **Null** site = 18.1, year = 11.6, residual = 33.9 **i_24hr** site = 11.2, year = 1.6, residual = 34.5 **i_Day** site = 13.1, year = 4.0, residual = 34.7 **i_Night** site = 11.3, year = 2.2, residual = 34.1 **latvar_24hr** site = 12.2, year = 4.7, residual = 34.6 **latvar_Day** site = 15.2, year = 8.4, residual = 34.5 **latvar_Night** site = 11.7, year = 4.1, residual = 34.2.

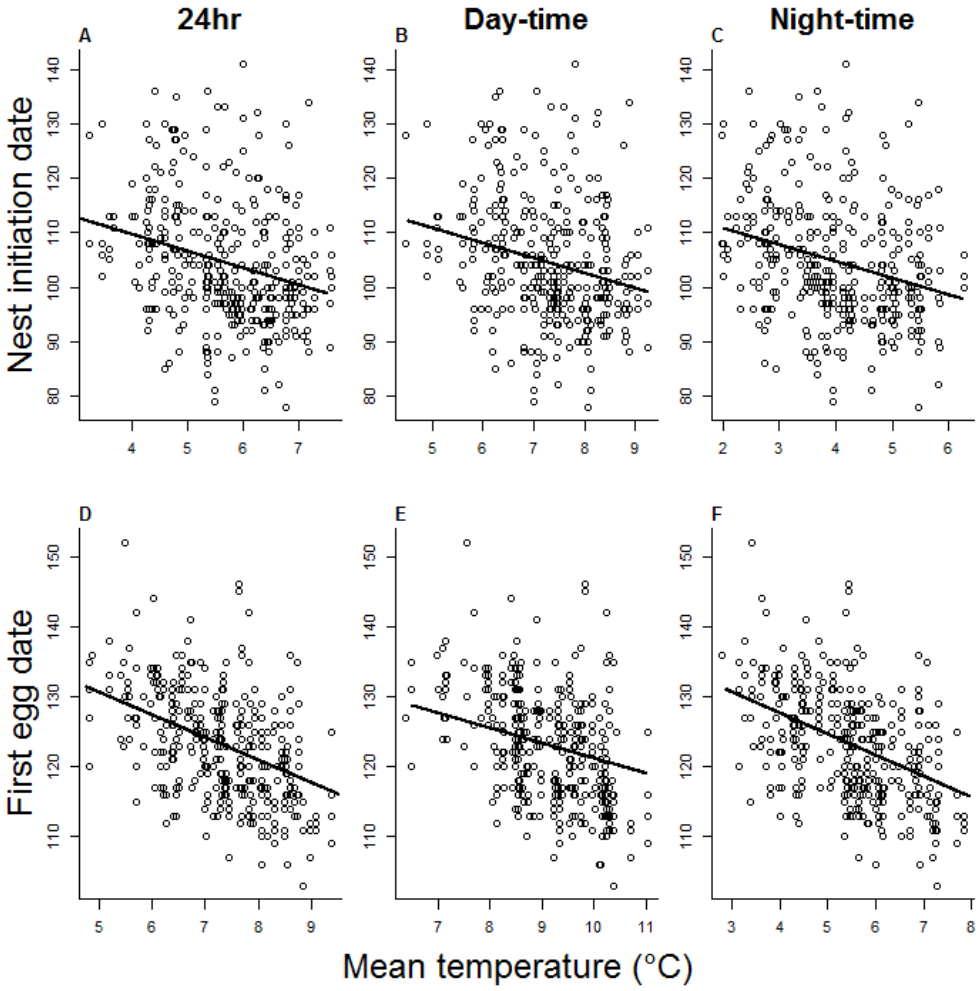

Figure 3.1 A-C: Relationships between mean temperature during the period temp_sw and N1: **A** showing overall mean temperatures during this period **B** mean day-time temperaures **C** mean night-time temperatures. **D-F:** Relationships between mean temperature during the period temp_i and FED are shown: **D** overall **E** day-time **F** night-time. All slopes taken from LMM's summarised in Table 3.2.

3.4.2 Tree phenology as a predictor of blue tit reproductive phenology

The slopes of all models using tree phenology as a predictor of blue tit reproductive phenology revealed that later tree phenology always predicts later reproductive phenology. Whilst this slope was non-significant for N1, both first bud burst (FBB) and first leaf (FLF) were significant predictors of the first egg date of blue tits, but first bud burst was both a stronger predictor and had a lower AIC and was thus the best tree phenology predictor of FED (Table 3.3, Figure 3.2). The models predict that when mean first bud burst at a site occurred on day 100, first egg date is predicted to occur on day 122, whilst a mean first bud burst of day 140 would predict a first egg date of day 131. None of the best linear unbiased

predictors for the random regression across individual tree genera differed significantly (i.e. a credible interval departing from 0) from the general tree phenology slope in any of the three models.

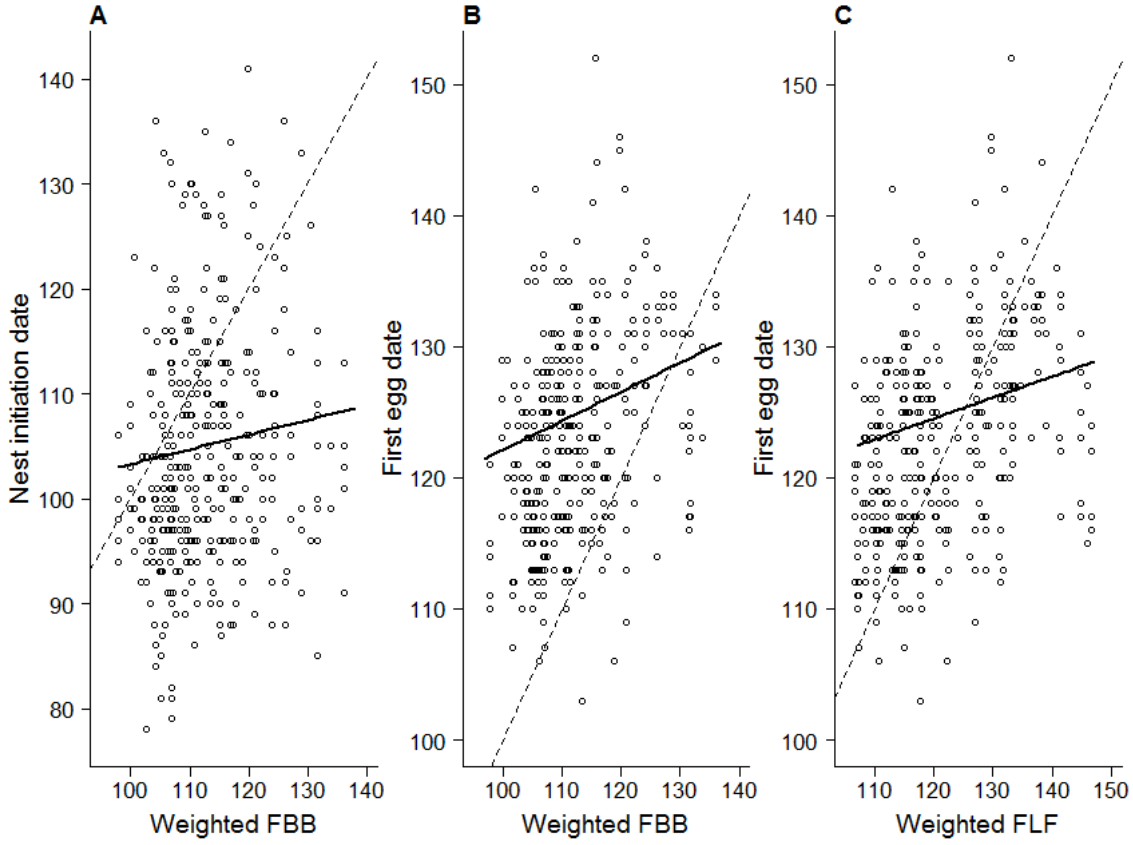

Figure 3.2 Relationship between **A** N1 and mean weighted FBB **B** FED and mean weighted FBB **C** FED and mean weighted FLF. Solid lines depict the predicted slopes given from LMM's in Table 3.3 and the 1:1 relationships are shown by hashed lines.

Table 3.3 Tree phenology predictors of N1 and FED, with their slopes (b) and upper and lower 95% credible intervals in brackets. Bayesian p values are reported for inferring significance and DIC for model comparison.

	Predictor	Intercept	b	Trees	pMCMC	DIC
N1	FBB	89.3 (62.6-115.3)	0.14 (-0.06-0.35)	0.00 (0.00-0.00)	0.19	3117.7
FED	FBB	100.0 (81.6-122.4)	0.22 (0.08-0.36)	$5x10^{-4}$ ($2x10^{-7}$-$1x10^{-3}$)	0.004^{**}	2333.8
	FLF	105.4 (87.0-126.1)	0.16 (0.04-0.29)	$3x10^{-4}$ ($1x10^{-8}$-$1x10^{-3}$)	0.016^{*}	2340.2

Random effect variances for **N1** model were: **FBB** site = 24.8, year = 189.4, residual = 97.1. Random effect variances for **FED** models were: **FBB** site = 11.6, year = 255.9, residual = 33.5 **FLF** site = 11.4, year = 141.0, residual = 34.2.

3.4.3 Invertebrate abundance as a predictor of blue tit reproductive phenology

Using sliding windows I found the best mean invertebrate availability predictors of N1 and FED were between dates 82 and 95 (23rd March – 5th April in a non-leap year) for N1 and dates 93-146 (3rd April – 26th May in a non-leap year) for FED. Models that included invertebrate availability outperformed the null models (Table 3.4). However, the effect sizes were small, such that N1 was predicted to occur on day 105 when invertebrate availability was at its lowest value and just four days earlier when invertebrate availability was at its highest value, whilst FED was predicted to occur on 128 when invertebrate availability was at its lowest value and nine days earlier when at its highest value (Figure 3.3).

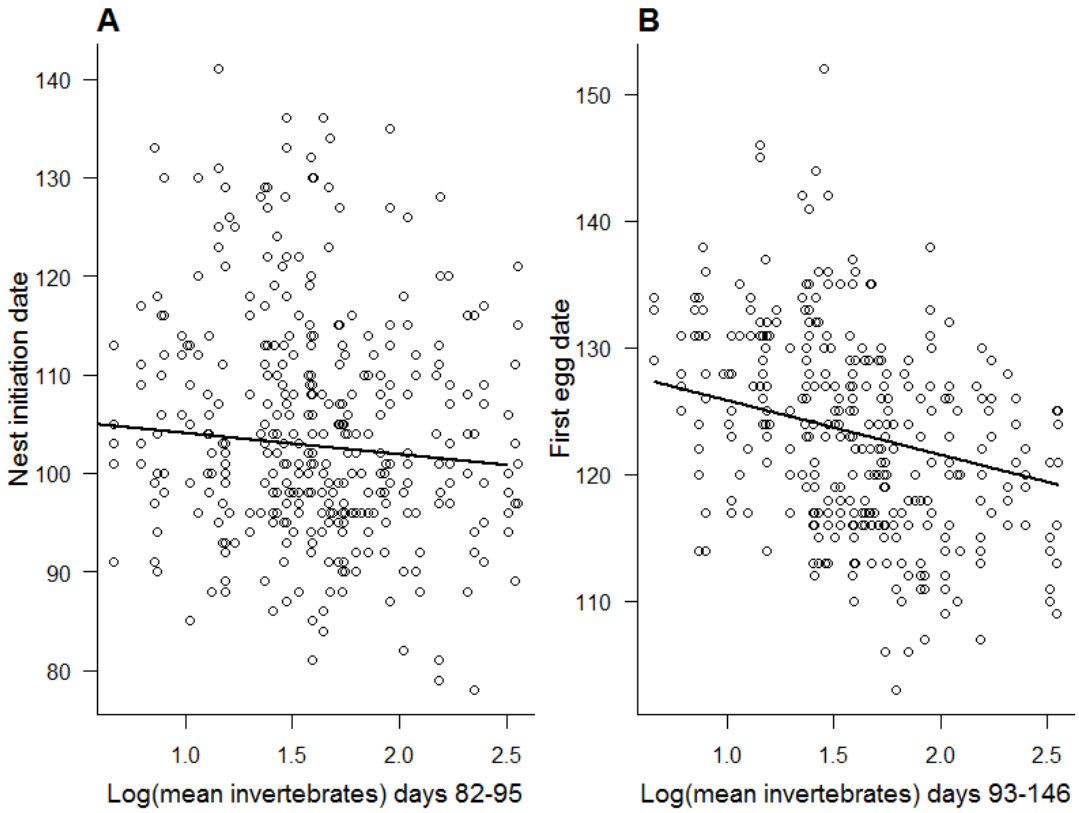

Figure 3.3 Relationship between **A** N1 and mean invertebrate availability in the period of days 82-95 **B** FED and mean invertebrate availability in the period of days 93-146. Slopes shown are from the best invertebrate predictor LMM's summarised in Table 3.4.

Table 3.4 Invertebrate abundance predictors of N1 and FED, with slopes (b) and associated standard errors (se) taken from LMM's (see methods), along with null models and AICs for comparison.

Response	Start Date	Duration	End Date	Intercept ± se	b ± se	AIC
N1		Null		104.5± 1.4		3145.6
	82	14	95	106.2 ± 1.8	-2.16 ± 1.56	3106.5
FED		Null		123.3 ± 2.1		2350.2
	93	54	146	130.2 ± 2.8	-4.31 ± 1.43	2344.2

Random effect variances for **N1** models were: **Null** site = 28.3, year = 3.0, residual = 96.2 **Invertebrates** site = 24.8, year = 2.4, residual = 98.2. Random effect variances for **FED** models were: **Null**: site = 17.3, year = 11.3, residual = 34.3 **Invertebrates**: site = 14.4, year = 6.3, residual = 34.2.

3.4.4 Combined predictors of blue tit reproductive phenology

In the full models, that included the best predictor from each single predictor model and latitude as a proxy for photoperiod, there is general agreement on the significance and slope of each predictor variable throughout the different models (Full, Null, -Multi, spaMM) for each phenological response, N1 and FED (Table 3.5). N1 is significantly predicted only by the best temperature predictor, night-time temperature during the period temp_sw, with the other predictor variables unimportant in predicting when N1 will occur (Table 3.5).

In comparison, FED was significantly, or marginally significantly, predicted by both the best temperature predictor, night-time temperatures during the period temp_i, and mean invertebrate availability during days 93-146. Tree phenology and latitude were both non-significant predictors of FED.

When comparing the variances between the null and full models (Table 3.5 footnotes), approximately one-third of the inter-site variance and over half of the inter-annual variance in N1 was explained by the N1 full model, whereas around half of the inter-site and over two-thirds of the inter-annual variance in FED is explained by the FED full model. Spatial autocorrelation from the spaMM models is negligible, with the range at which autocorrelation drops to 0.1 being 0.009° for N1 and 0.008° for FED, both equating to distances well within a single site (0.05 of a site).

Table 3.5 Summarising the model outputs from LMM's (described in 3.3.6) incorporating all predictors of N1 and FED. Null, Full and –Multi models show slope estimates for each predictor with a p value and significance asterisk in brackets, whilst the spaMM models show slope estimates ± standard errors. Temperature shows the slope for the best temperature predictor found for each response in Table 3.2 (temp_sw_night for N1, temp_i_night for FED), tree phenology shows the slope for the best tree phenology predictor for each response in Table 3.3 (weighted FBB for both), invertebrate availability shows the slope for the best invertebrate availability predictor for each response in Table 3.4 (mean availability between days 82-95 for N1, days 93-146 for FED) and photoperiod shows the slope for latitude as a proxy for photoperiod.

Response	Model	Intercept	Temperature	Tree phenology	Invertebrate availability	Photoperiod
N1	Null	104.4				
	Full	149.6	-2.75 (0.02*)	0.05 (0.63)	-1.05 (0.52)	-0.67 (0.69)
	-Multi	142.7	-2.74 (0.01*)	0.06 (0.57)	-0.93 (0.57)	-0.58 (0.74)
	spaMM	137.9	-2.85 ± 0.71	0.03 ± 0.09	-0.94 ± 1.53	-0.44 ± 1.67
FED	Null	123.1				
	Full	205.3	-1.87 (0.06)	0.11 (0.16)	-2.86 (0.05)	-1.39 (0.26)
	-Multi	175.9	-2.31 (0.00**)	0.08 (0.32)	-3.17 (0.03*)	-0.77 (0.51)
	spaMM	176.9	-2.39 ± 0.57	0.07 ± 0.07	-3.39 ± 1.33	-0.76 ± 1.11

Random effect variances for **N1** models were: **Null** site = 31.1, year = 99.4, residual = 98.5 **Full** site = 23.1, year = 38.5, multi-membership = 0.0002, residual = 98.8 **–Multi** site = 25.5, year = 38.5, residual = 98.6 **spaMM** site = 27.5, year = 0.00, nu = 0.5, rho = 267.3. Random effect variances for **FED** models were: **Null** site = 19.1, year = 220.8, residual = 34.5 **Full** site = 9.6, year = 67.9, multi-membership = 0.0003, residual = 34.1 **–Multi** site = 11.5, year = 69.3, residual = 34.6 **spaMM** site = 12.7, year = 1.0, nu = 0.5, rho = 287.6.

3.5 Discussion

Average night-time temperature in early spring was the most important predictor of blue tit breeding phenology, with elevated night time temperatures significantly predicting earlier nest initiation and lay date across sites and years. High invertebrate abundance also significantly predicted earlier lay date but not nest initiation. Tree phenology and latitude were found to be non-significant predictors in a multi-predictor model, despite tree phenology significantly predicting lay date when considered individually. Day-time temperatures were inferior predictors of blue tit breeding phenology compared with night-time temperatures. These results concur with previous studies suggesting that temperature is a strong causal predictor of lay dates in woodland passerines (Visser *et al.* 2009; Phillimore *et al.* 2016). However, this finding advances our understanding of temperature's role as an environmental predictor of blue tit reproductive phenology as this study is the first to report

differential impacts of night- and day-time temperatures. By considering nest initiation alongside the more traditional laying date I extend our understanding of the predictors of phenology earlier in the nesting period. I show that increased natural food availability advances lay dates, advancing previous research that shows that supplementation of diet with artificial food can advance lay date (Svensson & Nilsson 1995; Nager *et al.* 1997). However, I find no support for previous results suggesting that tree phenology is an important environmental predictor of woodland passerine breeding phenology (Nilsson & Källander 2006; Bourgault *et al.* 2010) and suggest that this correlation may occur due to co-variance with temperature.

Spring temperatures have long been known to be a strong negative correlate of woodland passerine laying dates, with some experimental studies reporting a direct effect (Visser *et al.* 2009). This hypothesis receives some support here, as temperature effects were stronger than any other predictor tested. The slope found from the best temperature periods of around three days earlier bird phenology per 1°C increase is comparable to those found in other studies (Husby *et al.* 2010; McLean *et al.* 2016; Phillimore *et al.* 2016), albeit slightly shallower than some, and was consistent between nest initiation and lay date. This is the first study to identify night-time temperatures as more important than overall (or day-time) temperatures as predictors of breeding phenology. I suggest that minimum temperatures are therefore likely to be more important in timing woodland passerine reproduction than maximal temperatures, which is in agreement with some previous research suggesting that cold night time temperatures act as a constraint on egg-production due to the energetic costs of producing and incubating eggs (Yom-Tov & Wright 1993; Stevenson & Bryant 2000) and somewhat challenges the idea of increasing temperatures acting as a cue (Visser *et al.* 2009; Schaper *et al.* 2012). Another possible explanation for the finding that night time temperatures are important is if night-time temperatures are a better predictor of invertebrate availability than our estimates, and this in turn affects breeding phenology. One advantage of finding that temperature is a reliable predictor is that temperature is easy to measure and this lends itself to predicting how populations will respond in the future (Vedder, Bouwhuis & Sheldon 2013).

The periods during the spring when I find blue tit phenology to be most sensitive to invertebrate availability, despite being estimated from a fairly flat likelihood surface, were also credible, with an earlier period (the earliest possible start given the data available) for nest initiation than lay date. Although a significant predictor of both measures of

reproductive phenology when tested individually, invertebrate abundance was only a significant predictor of lay date in the full models. Across the range of observed invertebrate abundances the predicted effect size was a nine day difference in lay dates and this is highly similar to the difference shown by artificial feeding in other studies (Svensson & Nilsson 1995; Nager *et al.* 1997). This may reflect the maximum amount that females will plastically shift laying due to food availability. However, our estimates of food available to the blue tits may be an inaccurate estimate of true blue tit diet, by missing non-flying invertebrates and due to the variability inherent in catching insects on sticky traps. Thus, I cannot rule out the possibility that average nightly temperature may actually be a better predictor of possible prey abundance than our estimate, as minimum temperatures are known to affect invertebrate growth and availability (Petavy *et al.* 2001; Bale *et al.* 2002). If this were the case it is possible that invertebrate availability is actually more important than recognised here.

Tree phenology was always positively correlated with bird phenology in single predictor models, but the effect was diminished and non-significant in the full multi-predictor model, contrary to the findings of some other studies (Thomas *et al.* 2010; Bourgault *et al.* 2010). The most significant single predictor tree phenology model predicted that one day later first bud burst gave 0.22 days later lay date. This effect was greatly reduced in the full model, presumably being accounted for by its close correlate, temperature (Polgar & Primack 2011). Perhaps it is unsurprising that first bud burst did not predict nest initiation and first leafing did not predict first egg date well, as the respective tree phenology sometimes developed later than the respective bird phenology, and therefore logically cannot be a cue (Figure 3.2), unless bud burst is a predictor of an earlier phase of tree phenology, such as bud swelling. The phenology of none of the individual tree genera significantly improved the predictive power of these models, implying that the birds are not responding to any tree genus above any other, as has been suggested for oak and birch previously (Nilsson & Källander 2006).

Previous research has demonstrated that photostimulation is fundamental in commencing temperate passerine reproductive phenology (Lambrechts & Perret 2000; Helm *et al.* 2013), but I found no evidence that it explains the spatial and temporal variation observed on the scale of this study. This supports the idea that photostimulation opens a ‘window’ for possible breeding beyond which other supplementary cues, noted above, refine the exact timing, and these processes give rise to the variation that we observe (Lofts & Murton 1968; Dawson *et al.* 2001). However, the direction of the slope does suggest that the north of the transect tends to initiate reproductive phenology before the south once all else is controlled

for, which would be consistent with slight photoperiodic involvement after the equinox, as day length is longer and increases faster the further north.

The insights gleaned from this study could be interpreted as a support for predictors constraining blue tit reproductive phenology rather than cuing it. Night-time temperatures receiving greater support than day-time temperatures suggests that minimum temperature is more important than maximum and that increasing temperatures affect timing through lifting energetic constraints rather than signalling approaching favourable conditions. Although a novel result in itself, this concurs with the existence of a thermal constraint on egg laying (Yom-Tov & Wright 1993; Stevenson & Bryant 2000), which is highly energetically costly for the female (Perrins 1970), and also helps explain why female yolk development (Caro *et al.* 2009) – but not male gonadal development (Caro *et al.* 2006) – correlates with laying dates. Nest initiation also responded to night-time, and therefore more likely minimum than maximum, temperatures, suggesting that this may also be energetically costly and constrained. If energetic constraints are a major factor influencing blue tit egg formation and lay dates, increasing food abundance advancing this process also makes biological sense in this context (Perrins 1970).

Using spatial replication of field sites rather than traditional single-site studies to analyse these questions captures substantial independent variation in each of the putative drivers within each year, making it easier to tease apart their separate effects. It also provides greater variation of leafing phenology due to the different habitats dominated by different tree genera. A limitation, however, is the lack of precision and replication at each individual site due to practical restrictions, and the limited number of years over which this study was conducted, which may be an issue if the slope in response to predictors is different over time to that over space, or if conditions vary more between years than locations (Dunne *et al.* 2004).

This study also highlights the importance of modelling all potential predictors together when answering this question, as some that are significant when modelled individually (e.g. tree first bud burst effecting lay date) lose importance when included in a general model through their correlation with another, more influential factor (e.g. temperature). Both full models explain a large proportion of the geographical and annual variation inherent in this system, lending confidence to their inferences. One future direction to test this result could be to

analyse the effects of minimum and maximum temperature on lay dates under controlled laboratory conditions with *ad lib* feeding.

In summary, average night-time temperatures (over different periods in early spring) were significant predictors of both nest initiation and lay date in Scottish blue tits, while invertebrate availability also significantly predicted lay date but not nest initiation. Tree phenology and photoperiod were unimportant in explaining the variation observed in either. Previous research has often speculated that the effect of temperature on lay date may be indirect, via its effect on tree phenology, or invertebrate abundance, but including all of these putative drivers in a single model and temperature emerging as the most important predictor lends support to the hypothesis that it has a direct effect over and above the other factors. These results contribute to our understanding of which factors schedule insectivorous woodland passerines reproductive phenology, allowing for more accurate predictions of how future climate change will affect this and influence potential trophic mismatches (Lyon *et al.* 2008).

Chapter 4

Faecal metabarcoding derived insights into spatio-temporal variation in blue tit diet prior to breeding

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Aviemore

4.1 Abstract

Temperate insectivorous passerines breed earlier in warmer years, but it is unknown whether the birds respond directly to temperature or whether they respond to a change in diet, which is itself cued by temperature. The lack of high resolution prey data has made addressing this question very difficult. In addition, how the diet of a generalist insectivore varies geographically and temporally is poorly understood. To address this, I collected 959 faecal samples from nestbox-roosting adult blue tits (*Cyanistes caeruleus*) at 35 sites along a 220km transect of Scotland throughout the springs of 2014-15 prior to breeding. 793 faecal samples were metabarcoded at the cytochrome oxidase I (COI) genetic marker to identify the presence of prey DNA, along with 24 controls of three types (extraction negatives, PCR negatives and positives) and 30 repeat samples (from two halves of faecal samples) to assess contamination and repeatability in the faecal metabarcoding method. A mean of 5.1 prey taxa were recorded per sample. In total 432 total prey taxa were identified from 18 invertebrate orders, with six orders dominant and *Lepidoptera* the commonest of these, being present in 73.6% of samples. I present a new approach for estimating trends in α- and β-diversity across sites and along continuous gradients from a mixed model. Blue tit dietary α-diversity increased throughout the spring, but was not significantly correlated with elevation or latitude. Turnover in the species comprising diet (dietary β-diversity) was pronounced, with high levels of site-to-site dietary differentiation and significant turnover found across latitude, elevation and date, with latitudinal turnover the least pronounced. In addition, a substantial increase in *Hemiptera* and *Lepidoptera* in the diet pre-laying could represent a possible dietary cue to reproductive phenology. Repeatability in the identification of a specific taxon in a faecal sample was fairly high (0.59). This study reveals how careful application of next generation sequencing methods can provide highly resolved and novel insights into the diet of even intensively studied avian species, such as the blue tit.

4.2 Introduction

Insectivorous passerines, such as tits (*Paridae*) and flycatchers (*Muscicapidae*), breeding in temperate woodlands have become a model system for studying a wide range of ecological questions, including the impacts of trophic mismatch (Visser *et al.* 1998; Both *et al.* 2006). A key unresolved aspect in trophic mismatch is how the passerines time their reproductive phenology (e.g. egg laying) and the environmental predictors that they utilise (Bourgault *et al.* 2006; Källander *et al.* 2017). Whilst it is known that spring temperatures correlate with egg laying (Visser *et al.* 2009; McLean *et al.* 2016; Phillimore *et al.* 2016), it is unknown whether this is a direct effect or mediated by a correlated factor such as food abundance or a specific dietary item acting as a cue (Caro *et al.* 2013). This is largely due to difficulties in identifying adult passerine diet over a long temporal scale, with large sample sizes and to detailed taxonomic resolution. Molecular techniques, such as faecal metabarcoding, whereby small fragments (minibarcodes) of prey DNA are amplified and identified from faecal matter, could provide a solution (Pompanon *et al.* 2012; Taberlet *et al.* 2012; Clare 2014a).

Tits are known to rely heavily upon *Lepidoptera* caterpillars for feeding nestlings during the breeding season (Nour *et al.* 1998; Wilkin *et al.* 2009; Cholewa & Wesołowski 2011), with the winter moth (*Operophtera brumata*) especially important (Perrins 1991; Visser *et al.* 1998). Much less is known about adult diet and how this varies temporally and geographically. There is a much higher degree of understanding for nestling diet due to the relative ease of sampling, as videos and cameras placed near the nest to record prey items brought, and neck collars on nestlings allow direct sampling (Blondel *et al.* 1991; Arnold *et al.* 2010; Burger *et al.* 2012). However, adults often forage in hard to observe locations and this combined with small prey size make visual observation challenging (but see Gibb 1954). Traditionally, therefore, investigation of adult diet has required euthanising large numbers of birds and dissection and microscopic analysis of gizzard or gut contents (Betts 1955; Sehhatisabet *et al.* 2008). Not only does this method require destructive sampling of the study species, it precludes the identification of soft-bodied dietary items, cannot provide a time-series, and often yields only relatively poor taxonomic resolution (e.g. order or family level). The advancement of next-generation sequencing and faecal metabarcoding now enables non-destructive and high-resolution dietary sampling (Symondson 2002; Pompanon *et al.* 2012; Taberlet *et al.* 2012), revealing prey items consumed recently before defecation (Oehm *et al.* 2011) and with close correlation to morphological methods and known diet supporting the accuracy of faecal metabarcoding (Deagle & Tollit 2007; Zeale *et al.* 2011).

The method is particularly effective for identifying invertebrate prey, due to the rapidly evolving cytochrome oxidase subunit 1 (COI) mitochondrial gene, which is accepted as a reliable standard and allows identification resolution to species-level in most cases (Clare 2014a; Kress *et al.* 2015).

Seasonal variation in tit diet appears to be substantial (Betts 1955; Cramp & Perrins 1993). Temporal dietary turnover may arise either via fluctuations in prey preference or as a result of phenological changes in the availability of different prey and could provide a cue to commence reproductive phenology, as has been hypothesised in another bird species (Barea & Watson 2007). For example, it has been shown that female insectivorous passerines change their diet whilst egg laying to incorporate more calcium-rich items for egg formation (Graveland & Berends 1997; Bureš & Weidinger 2003). Betts (1955) provides the most comprehensive insights to pre-breeding diet, based on analyses of gut contents, and reports that in March *Hemiptera* are found to be the commonest prey of blue tits (*Cyanistes caeruleus*), switching to *Dipteran* larvae in April, with *Hymenoptera* and *Lepidoptera* present at lower levels throughout this period, such that a dietary rise in any of these prey orders could plausibly act as a cue.

Additionally, Betts (1955) and Sehhatisabet *et al.* (2008) highlight the generalism of tit diet and the contribution of several invertebrate orders to the diet, including *Hemiptera*, *Diptera*, *Coleoptera*, *Hymenoptera* and *Araneae* alongside the better-known *Lepidoptera* and winter plant matter. This hints at dietary flexibility, though the taxonomic resolution of prey and geographic spread of data is currently insufficient to assess the degree of geographic turnover in adult tit diet. However, nestling diet is known to vary with habitat, with higher dietary proportions of caterpillars in deciduous woods than coniferous (Gibb & Betts 1963; Burger *et al.* 2012) or sclerophyllous (Blondel *et al.* 1991; Bańbura *et al.* 1994), and even proximity to certain tree species influencing nestling diet (Wilkin *et al.* 2009). Indeed, how complex food-webs and the diet of generalist predators vary geographically is poorly understood and the degree to which food webs change along major environmental gradients (e.g. elevational, latitudinal, temporal) is unclear. Although we are aware that generalist species can utilise a variety of prey resources (Burger *et al.* 2012; Sedlock, Krüger & Clare 2014), we are unaware of the magnitude of the changes along these environmental axes. If changes in trophic interactions between environments are large this implies substantial differences in food web structure and could have profound effects determining local community ecology in general and on trophic mismatch (e.g. dietary cue, species

mismatched to) in this system. High dietary variability could imply that if a dietary cue is used and similar across populations, that this cue would need to be at a high taxonomic level as the precise prey species in the diet are highly geographically variable.

Alpha- (α, total species diversity at a particular site/unit) and beta- (β, site-to-site variability in community composition) diversity allow quantification of ecological richness and distinctiveness (Yu *et al.* 2012; Kress *et al.* 2015). Environmental metabarcoding has been demonstrated to allow precise estimation of α- and β-diversity (Yu *et al.* 2012) but this ability has not yet been extended to faecal metabarcoding. As invertebrate α-diversity is known to generally show a negative relationship with latitude, elevation and temperature (Gaston & Williams 1996; Wilf & Labandeira 1999; Bale *et al.* 2002), this has the potential to subsequently affect blue tit diet. Quantification of these measurements would identify the environmental drivers of diet variation (dietary richness and turnover) in a generalist insectivore, such as the blue tit.

Thus far, faecal metabarcoding has predominantly been utilised to elucidate the diet of mammals (Quéméré *et al.* 2013; De Barba *et al.* 2014), particularly bats (Clare *et al.* 2009; Bohmann *et al.* 2011). The method has been used to address questions regarding seasonal and inter-annual dietary variation (Clare, Symondson & Fenton 2014b), locational and habitat dietary variation (Clare *et al.* 2011, 2014a; Quéméré *et al.* 2013) and interspecific niche partitioning (Bohmann *et al.* 2011; Razgour *et al.* 2011; Sedlock *et al.* 2014). Relatively few avian faecal metabarcoding studies have been published, with chemicals present in avian faeces presenting a challenge to the application of these methods (Jedlicka, Sharma & Almeida 2013; Vo & Jedlicka 2014). In general, faecal metabarcoding studies have had limited sample sizes, with all avian studies to date containing (often far) fewer than 80 independent samples, mostly sampled from a single location and often comprising multiple bird species (Coghlan *et al.* 2013; King, Symondson & Thomas 2015; Crisol-Martínez *et al.* 2016). The only avian geographic dietary comparison using faecal metabarcoding to date found similar dietary composition in Louisiana waterthrush (*Parkesia motacilla*) nestlings at two distant sites (Trevelline *et al.* 2016). Whereas in western bluebirds (*Sialia mexicana*) adult and nestling diets differed but time of spring had no significant effect (Jedlicka, Vo & Almeida 2017).

Repeatability has rarely been quantified in faecal metabarcoding studies, but where it has been tested it has been found to be high once beyond the PCR stage and in the sequencer (De

Barba *et al.* 2014) but much lower when faeces are initially subsampled with separate DNA extractions (Jedlicka *et al.* 2017). This suggests that what is extracted from the faecal sample could vary depending on the part of the sample used but that the contents of this extraction will amplify consistently. Positive controls to ensure accuracy and negative controls to examine contamination are even rarer in faecal metabarcoding studies, with the only example I am aware of reporting no contamination and reasonably reliable throughput of known sequences (De Barba *et al.* 2014). Amplification biases have been noted (Clarke *et al.* 2014), however the large range of invertebrate prey taxa found in all studies (Clare *et al.* 2014a; Trevelline *et al.* 2016) and the concordance with known diet (Zeale *et al.* 2011; Groom *et al.* 2017) support the general reliability of the approach.

This study employs faecal metabarcoding to resolve the diet of an insectivorous woodland passerine, the blue tit, in early spring along a 220 km transect of Scotland. The main aims of this study are three-fold. First, I will use a novel approach examine the effects of time of year, latitude and elevation on dietary α- and β- diversity of a generalist predator. I also address this question at the order level, focusing on trends in the presence of major prey groups. My second aim is to test whether there is any signal that is consistent with a dietary cue for reproductive phenology. Specifically I test whether the species richness of particular prey groups increases in the run up to the timing of mean egg laying at a site. Third, I aim to improve the methodology of faecal metabarcoding in adult bird samples and assess the accuracy of the faecal metabarcoding technique, specifically with regards to repeatability and contamination which have been under-examined to date.

Box 4.1 Explanation of dietary α- and β- diversity, as used in this study

Diet can vary both in the number of taxa consumed, and in the identity of those taxa. Traditional alpha- (α-) diversity describes the species richness of a place (Whittaker 1972). In this study, I define dietary α-diversity as the species richness of a diet i.e. how many different prey taxa are consumed at a certain location and time. Dietary α-diversity may then vary dependent on location, biogeographic or environmental variables, or time of year. Traditional beta- (β-) diversity concerns the extent of species replacement or community differentiation along environmental gradients (Whittaker 1972), and measures the turnover of species between sites. In this study, I define dietary β-diversity as the turnover of dietary species between locations or across environmental gradients i.e. to what degree turnover is occurring in the identity of consumed taxa between locations or across environmental gradients. This is achieved by measuring changes in species' probability of occurrence along a gradient and estimating how much the trends in occurrence along the gradient vary, with high variance equating to a high degree of turnover and thus high dietary β-diversity.

4.3 Methods

4.3.1 Collection of field data and samples

Data were collected during the springs of 2014-15 from 39 predominantly deciduous woodland sites comprising a 220km transect in Scotland along a roughly north-south axis (see section 2.3.1 and Table 2.1). Six Schwegler 1B 26mm-hole nestboxes were available at each site at approximately 40m intervals and the floor of each nestbox was covered with greaseproof paper (for greater DNA retention – see Oehm *et al.* 2011) from mid-March each year until the onset of nesting in that nestbox (N1 – see section 3.3.5), whereupon it was removed. Each nestbox was checked every other day throughout this period and any faeces on the greaseproof paper removed with sterilised tweezers (after use they were wiped with lab tissue and then treated with ethanol and fire) and up to a maximum of three were collected in an Eppendorf pre-filled with pure ethanol. The remainder were discarded and the number of faeces collected recorded. The greaseproof paper was replaced when it became dirty, wet or damaged. In some instances the greaseproof paper was pulled half through the hole by the birds, and in these cases it was removed and not replaced so as to not discourage breeding. Samples were stored at -18°C within a day of collection and at the end of the field season were transferred to a -20°C freezer. Faecal samples were collected from 35 of the 39 field sites (see Table 2.1 – sites with no samples were EDI, DNC, DNS and RTH).

Latitude and elevation were obtained for each nestbox as described in 2.3.1 and the habitat survey protocol and derivation of habitat parameters are described in 2.3.2. Bird phenology (nest initiation (N1) and first egg date (FED)) was recorded as is described in 3.3.5. In 2014 at each of the 30 sites studied in that year (see Table 2.1) 10 waxworms (*Galleria mellonella*) were provided every two days in a plastic cup attached to the same tree as two of the nestboxes until the first egg had been laid. The aim of this supplementary feeding experiment was to understand the role that food availability plays in breeding phenology. However, subsequent statistical analysis revealed that the treatment had no effect on FED.

4.3.2 Molecular labwork

793 of the 959 total collected faecal samples were subsampled, placing an upper limit of 10 samples per nestbox per year. Where the limit of 10 was exceeded the subsampling was designed to maximise the range of dates on which a nestbox was represented. If multiple faeces (2 – 3) were present within a sample tube, part of each faeces was used for the DNA

extraction in order to sample a broad range of the respective bird's diet within the previous day. In addition, 30 samples were processed in duplicate to test the repeatability of the metabarcoding process and these were evenly distributed throughout the sampling period, including samples from multiple sampling locations in both 2014 and 2015. The faeces for each of the 30 replicated samples were evenly divided into two and DNA extractions were performed on each replicate; each extraction was subsequently treated as though it were an independent sample for the remainder of the molecular and informatics protocols. All aspects of the following laboratory protocol (DNA extraction, PCR amplification, PCR clean-up, sequencing on a MiSeq run) were performed at different times using different aliquots of reagents for the two replicates of each of the 30 replicated samples, in order to make them as independent as possible.

DNA was extracted from faecal samples using the QIAamp DNA Stool Mini kit, following the protocol for pathogen detection with a few custom modifications designed to improve yields (section C1). These included homogenisation of faecal samples in lysis buffer by shaking in a TissueLyser with a tungsten carbine bead, increased lysis times in the presence of additional Proteinase K, and use of larger buffer volumes. Three loci were subsequently targeted for amplification through PCR - the standard animal barcoding gene (cytochrome oxidase subunit I (COI)), a secondary barcoding gene to detect invertebrate prey DNA and confirm the faecal sample originated from a blue tit and no other hole-dwelling passerine (16S rRNA), and a standard plant barcoding gene (rbcL).

Given that DNA from dietary items is expected to be very degraded, the primers used (section C2) amplified a small region of each gene (184-220 base pairs, a minibarcode). Invertebrate primer sets were validated to ensure that they would amplify DNA from the expected range of invertebrate taxa (arachnids, isopods, nine insect orders). The plant primers used have previously been demonstrated to work well on nearly all higher plant groups (Palmieri, Bozza & Giongo 2009; Little 2014). The 16S primers also amplified avian DNA, so provided sequence data to confirm that the faecal sample was derived from a blue tit, the focal species.

Amplicons for each gene were generated using a two-stage PCR protocol for each faecal sample separately. The first amplified the target gene using locus-specific primers with a modification at the 5' end to incorporate part of the Illumina Nextera XT adaptor required for downstream sequencing. This PCR was repeated in duplicate for each locus, with

duplicates within loci pooled before the second PCR. The second round of PCR added the remainder of the Nextera XT adaptor to the ends of amplicons, including indices to provide unique labelling of amplicons from each sample. Amplicons derived from different loci but the same faecal samples were labelled with the same index combination. Each reaction from the second PCR was then cleaned up (removing salts, unincorporated primers, and any possible adaptor dimer) and normalised to the same concentration using SequalPrep Normalisation plates. Samples were eluted from each plate using the same aliquot of elution buffer, hence pooling them at the same time as eluting. Each plate-by-locus combination was quantified and equimolar amounts combined into a single pool.

Control samples were introduced at various stages of the molecular work. Six different negative controls were introduced when performing the DNA extractions (using all the same reagents as samples, but with no faeces added). These six extraction negatives were carried through the remainder of the molecular and informatics methods, to provide indication of any contaminants that may have been introduced during the molecular lab processes. A separate negative control was also included in each PCR plate (n = 9) containing pure water in place of DNA extract, as was a positive control containing a mix of template DNA from one known species of insect (*Dryocosmus israeli*) and one known species of plant (*Inga pezizifera*), neither of which occurs in Scotland. These PCR negatives and PCR positives and a small subset of samples were run on agarose gels before the PCR plate was taken through to the next stage of the protocol; the PCR was repeated for the whole plate if either the negative contained any evidence of an amplicon band or the positive lacked a band. These PCR negatives and positives were also carried through to the MOTU definition steps described later in 4.3.3. As the positive control contained known species, it additionally acted as a control to confirm that sample indexing (at the lab stage) and de-multiplexing of samples (at the informatics stage, see 4.3.3) had been performed correctly.

The final pool contained amplicons from three loci derived from between 275 and 278 faecal samples, inclusive of multiple controls and replicates. Three such pools were produced to accommodate all 793 samples, 30 replicates and 24 controls (9 x PCR positives, 9 x PCR negatives and 6 x extraction negatives). Amplicons within each pool were sequenced on an Illumina MiSeq, using 150 bp paired-end reads.

4.3.3 Initial informatics processing

Sequencing reads were initially de-multiplexed into sets corresponding to individual faecal samples using the index combinations present within the adaptor sequences. Reads were then de-multiplexed into sets corresponding to each locus using the locus-specific primer sequences present at the beginning of each read. Adaptor sequences, primer sequences and poor quality base calls were then removed, leaving only sequence corresponding to the targeted gene regions. Subsequent processing of the sequences applied the Uparse pipeline (initially developed for 16S metabarcoding of bacteria) to data for each locus separately.

The first step in the bioinformatics pipeline was to merge the paired reads derived from either end of the sequenced fragment. This process was successful for all COI and rbcL reads and many 16S reads; 16S reads derived from avian DNA did not overlap, but comparison with known blue tit 16S sequences indicated that these reads could be combined by adding four "N"s between the forward and reverse reads to produce a composite sequence of the correct length (hereafter referred to as fused reads). Reads were then filtered to ensure that within a locus they were all of the same length; this process removed possible pseudogenes incorporating insertions/deletions. This set of filtered sequences was then used for two purposes. Firstly, the set of unique sequences present was determined, with counts made of their frequencies. Unique sequences represented by only a single read were removed as they most likely represent sequencing errors. The unique sequences were then clustered into molecular operation taxonomic units (MOTUs), grouping sequences together that had an identity of 98% or more. The most frequently occurring sequence within each MOTU was designated as the reference sequence for that MOTU. The second use of the filtered reads involved mapping them back to this reference set of MOTU sequences, allowing a mismatch of up to 2% between filtered reads and a reference sequence, to provide a more accurate assessment of the frequency of each MOTU within each faecal sample. However, the frequency of a particular sequence (amplicon) within the dataset may not necessarily reflect the frequency of the corresponding MOTU within an individual birds' diet, as amplicon frequency can be affected by the affinity with which the primers bind to template from different species. Hence the frequency of a MOTU was only used to provide a filter to remove the very low frequency MOTUs (that most likely reflect PCR-derived contamination) from the final set of MOTUs assigned to any particular faecal sample. The taxonomic identity of MOTUs was determined using a BLAST search of the reference set of

MOTU sequences against public databases (GenBank for 16S; GenBank and BOLD for COI and rbcL).

4.3.4 Quality control

Firstly, I tested whether samples were from blue tits by verifying the presence of blue tit fused16S sequences. The highest blue tit 16S read from the 24 control samples was 58 and as a precaution all faecal samples that yielded fewer than 100 blue tit 16S reads were excluded from further analyses as they were not conclusively confirmed to be blue tit faeces (n = 9). Of the remaining samples, blue tit was the commonest of the fused 16S MOTU in all but one sample, but this sample still had sufficient (n = 1465) blue tit reads for identity confirmation. No other avian DNA was present in any sample.

Secondly, COI reads were checked from control samples to confirm the presence of positive control species and provide a baseline for background noise. All nine PCR positive control samples contained MOTUs attributable to *Dryocosmus israeli* (range of reads = 7796 - 19115) and no more than 16 reads of any other MOTU identified as belonging to the Metazoan kingdom. Eight out of nine PCR negative controls contained no more than 19 reads of any MOTU. The ninth was highly contaminated, containing 6798 reads of more than 20 MOTUs. Therefore, I checked for systematic contamination visually by looking at Spearman's correlations in MOTUs between samples in neighbouring cells in the same PCR column or row within plates (Figure C1). The row containing the contaminated negative sample was found to have a substantially higher mean level of correlation (r = 0.37) than other row and column correlations (mean r = 0.04) (Figure C1). This was considered to be most likely a systematic contamination event and this row (n = 11 focal samples + 1 control) was removed from all analyses. In addition, closer inspection of the contaminated plate revealed two cells (both focal samples) in the neighbouring row to the contamination event containing very similar MOTUs with the contaminated row and these were also removed from further analysis due to suspected contamination. After excluding those samples, the MOTUs in the three most correlated rows and columns were then visually inspected to check for further systematic contamination, but no evidence for this was found. Of the six extraction negative controls, four contained no MOTU at a higher read frequency than 3. The remaining two contained contamination (maximum reads = 10037 and 1611) but on further inspection there was no evidence for this being systematic, but rather more random within a

plate. As there were few cases where a control had > 20 reads for any MOTU as background noise, I adopted 20 reads as the cut-off for identifying a MOTU as present in all samples.

These steps reduced the number of samples from 847 to 825 (772 focal) containing 2524 MOTUs. All MOTUs with fewer than 20 reads in any single sample were removed as probable false positives (remaining n = 1432 MOTUs). All MOTUs without any identification, or identified as environmental contamination, were removed (remaining n = 1323). Then, a full taxonomy was obtained for each remaining MOTU and taxonomic reduction of the dataset began to eliminate non-prey items. Firstly, only MOTUs belonging to the Metazoan kingdom were considered possible prey items (remaining n = 1078). Then, all MOTUs not belonging to the phyla Annelida, Arthropoda and Mollusca were discarded (remaining n = 1005). Finally, all mites in the dataset (of orders *Astigmata, Mesostigmata, Oribatida, Siphonoptera* and *Trombidiformes*) were removed, as they were likely to be ectoparasites rather than actively foraged prey (remaining n = 911). A MOTU identification percentage match quality cut-off of 90% was then determined as MOTUs below this threshold were identified as similar percentage matches to organisms of disparate phyla and classes (n = 785), as were all MOTUs identified as '*Arachnida* sp' as these MOTUs were mostly closely matched to fungi (probably contained within the original Arachnid specimen) (remaining n = 778). Taxa identified to an identification match of 90% or more are considered correct to a minimum of order level, and this is the level that is important to the analyses in this study. All *Dryocosmus* and *Galleria* (see details regarding feeding experiment in 4.3.1 and positive controls in 4.3.2) MOTUs were removed (remaining n = 757). Then, all remaining MOTUs were merged when belonging to the same best-hit taxon (remaining n = 432). Finally, the biological plausibility of *Lepidoptera* identifications was assessed, due to comprehensive UK occurrence data for this order (Sterling & Parsons 2012; Waring & Townsend 2017) and their importance to tit diet. Nineteen taxa were reassigned to a British species when this species was within a 1% match of the geographically implausible top hit. Taxa with a 99% or greater identity match with the identified BLAST hit are considered correctly identified to species level (Clare *et al.* 2009; King *et al.* 2015) and a histogram of identity matches is provided as Figure C2.

4.3.5 Statistical analyses

Analyses focussed on the presence/absence of taxa in a sample as read numbers are not considered a reliable measure of the amount of a taxon in a sample due to biases in binding

and amplification (Yu *et al.* 2012; Clare 2014a). Control samples were precluded from analyses due to containing either no or very few taxa. As 20 reads was considered a credible limit of background noise (see 4.3.4), all reads of 20 or less for a taxon within a sample were treated as MOTU absence. Because faeces were pooled, we would expect a positive relationship between the number of faeces in a sample (1-3) and the presence of a certain taxon in at least one and therefore this was controlled for by including number of faeces as a categorical fixed effect.

To assess repeatability, I used a Bayesian generalised linear mixed model (GLMM) (Hadfield 2010) with a threshold response testing for the presence or absence of each dietary taxon in each faecal sample. Fixed effects included year and the number of faeces in the sample (1-3), both as factors, with random effects including taxon, site, nestbox ID, PCR plate (to control for contamination), sample ID and all two-way interactions between taxon and the other random effects. Repeatability was assessed by the formulae below, with (i) within-sample repeatability, measuring the repeatability of identifying a particular taxon in the replicate sample if it was found in the corresponding focal sample and (ii) across-transect repeatability, measuring how much more similar samples are from the same faecal sample (i.e. the focal sample and its corresponding replicate sample) than they are with respect to the dataset as a whole.

$$\text{(i) } var_{\text{sample ID:taxon}} / (var_{\text{sample ID:taxon}} + var_{\text{residual:taxon}})$$

$$\text{(ii) } (var_{\text{site:taxon}} + var_{\text{nestbox:taxon}} + var_{\text{sample ID:taxon}}) /$$
$$(var_{\text{site:taxon}} + var_{\text{nestbox:taxon}} + var_{\text{sample ID:taxon}} + var_{\text{residual:taxon}})$$

The first analysis aimed at quantifying geographic, habitat and temporal variation in blue tit diet. The presence or absence of each taxon (n = 432) in each sample was used as the response variable in a Bayesian GLMM (Hadfield 2010) with a threshold error structure, which deals well with binary data, and parameter expanded priors. Year and number of faeces in the sample (1 - 3) were included as fixed effect factors, with ordinal date, latitude, elevation, total foliage, birch foliage, oak foliage and tree diversity as numeric fixed effects. These fixed effects quantify trends in dietary α-diversity. Site, nestbox and sample status (focal/duplicate) were included as random effects, along with site, nestbox, day in year, sample, PCR plate, column within plate and row within plate all individually interacted with taxon. Non-interacted terms were used to assess variation in dietary α-diversity while interacted terms assess variation in dietary β-diversity. I also included random slope terms

(and all covariances) to allow the ordinal date, latitude and elevation effects to vary across taxa, which provides an estimate of the magnitude of dietary β-diversity along particular gradients.

In a second analysis designed to understand how the most important prey orders vary in blue tit diet, the taxa were treated at taxonomic order level and the dataset reduced to the presence/absence in each sample of the six commonest orders (*Araneae*, *Coleoptera*, *Diptera*, *Hemiptera*, *Hymenoptera* and *Lepidoptera*), from now on termed 'focal orders' and together comprising over 91% of all prey taxa identified (see 4.4.1). A similar GLMM to that described in the first analysis was then set up to analyse the presence or absence of each focal order (n = 6) in each sample. Identical fixed effects were included, with the addition of focal order and date, latitude, elevation and tree diversity individually interacted with focal order. The same random effects were also included, but with the exclusion of column and row and the covariance matrix. All numeric predictor variables in both of the above analyses were scaled to have a mean of 0 and a variance of 1 to provide direct comparability of results and allow better mixing of models.

Finally, I attempted to ascertain whether there is a dietary cue signalling reproductive phenology. However, as faecal samples were not collected at every visit to each site this precludes application of standard approaches to detecting the effect of a driver on phenology (e.g. sliding-windows). Therefore, I modelled the frequency of different taxa in the diet in the days prior to egg-laying as a spline using general additive mixed models (GAMM) in the mgcv R package (Wood 2011). This model will not provide a direct test of whether an aspect of diet acts as cue, but functions as an exploratory tool to identify any changes in diet preceding egg-laying. Two response variables were considered (i) the number of constituent taxa of each focal order per sample treated as Poisson (log link) and (ii) the presence/absence of the same orders per sample treated as a binary (logit link). Fixed effects were year and the spline across days prior to the yearly mean first egg date (FED) at the site at which the sample was taken, with site and nestbox as random effects. Replicated samples were excluded from this analysis to avoid pseudoreplication. If the presence/diversity of a taxon were acting as a cue then we would anticipate that the spline should show an increase in the days preceding FED.

4.4 Results

4.4.1 Scottish blue tit diet in early spring

From 774 focal samples there were 432 prey taxa with 60.4% of these resolved to species level (> 99% identity match (see 4.3.4), Figure C2) and a mean ± sd of 5.06 ± 3.28 taxa per sample (maximum = 20 taxa, mode = 3 taxa). The majority of dietary taxa were uncommon, with a large proportion of taxa (42.4 %) only being recorded in a single sample and 74.3% recorded in five samples or fewer. Just 15 taxa were recorded in more than 50 samples, 11 of which were identified to species level (Figure 4.1), comprising four *Lepidoptera*, four *Hemiptera* and one each of *Collembola, Diptera* and *Coleoptera*. The four taxa recorded in over 50 samples not resolved to species level comprised two *Diptera* taxa, one *Hymenoptera* and one *Lepidoptera*. The most commonly recorded species, the *Lepidopteran, Argyresthia goedartella,* was found in 34.6% of samples. Winter moth, *Operophtera brumata*, an important dietary item for nestling tits (Perrins 1979; Wilkin *et al.* 2009) but not known from adult diet at this time of year, was found in 27 (3.5%) samples. A full breakdown of taxa identified in the diet can be found in Table C1.

Eighteen invertebrate orders were encountered in at least one sample, with *Lepidoptera* being both the most commonly recorded (present in 73.6% of samples) and taxon-rich (131 taxa) prey order recorded (Figure 4.2). However, other prey orders were also common, with *Insecta* dominating and orders *Hemiptera, Diptera, Hymenonptera and Coleoptera* recorded frequently. Outside *Insecta,* only *Araneae*, and to a lesser extent *Collembola*, were recorded fairly frequently. The other eleven prey orders were much less commonly found in the diet (Figure 4.2). The most taxon-rich families within *Lepidoptera* were *Geometridae* (27), *Noctuidae* (25) and *Tortricidae* (23) (Table C1). However, the most taxon-rich family in the diet was *Chironomidae* (*Diptera*) (Figure 4.2, Table C1), containing 31 taxa.

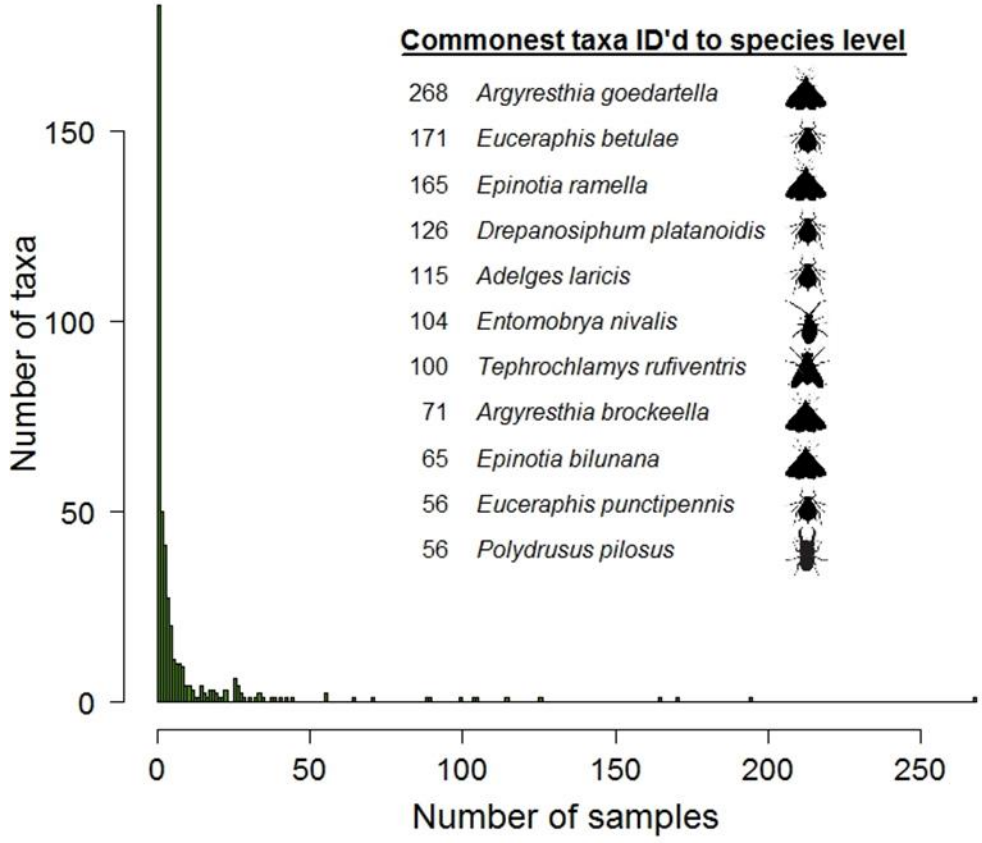

Figure 4.1 Histogram of the number of samples in which each taxon was found. Inset detailing the most prevalent taxa identified to species level (those recorded in more than 50 samples), with the number of samples they were recorded in and an order-level image of the taxon involved for ease of reference. See Figure 4.2 for image denotation.

4.4.2 Repeatability in faecal metabarcoding from separate DNA extractions of the same sample

Repeatability of the occurrence of a particular taxon in both a focal sample and in its corresponding replicate sample (within sample repeatability) was fairly high (mean = 0.59, credible intervals = 0.52 – 0.67). Repeatability when measured as similarity between the taxa identified in the two replicates compared to the dataset as a whole (across transect repeatability) was, as expected, even higher (mean = 0.78, credible intervals = 0.74 – 0.82).

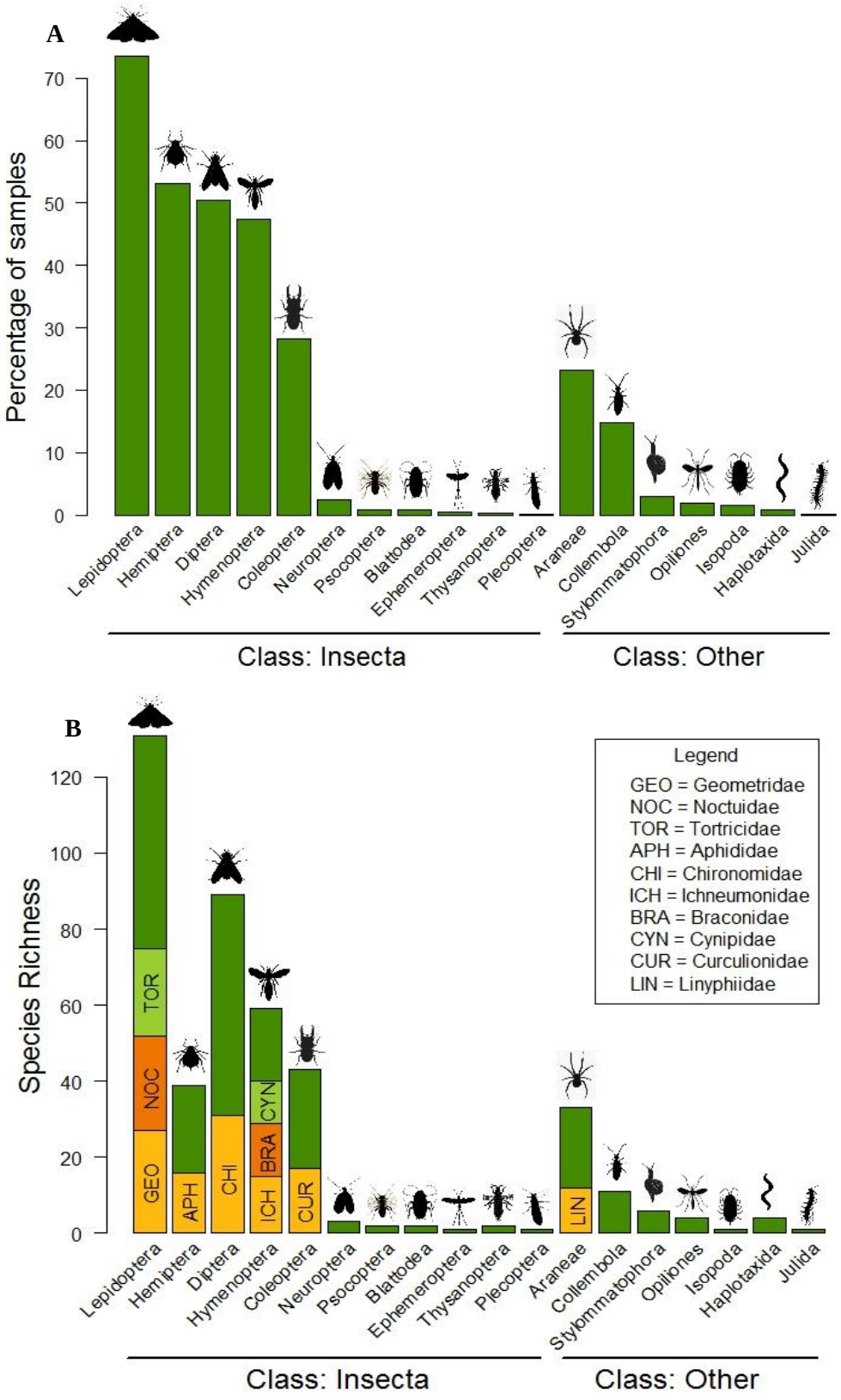

Figure 4.2 A Frequency of prey orders in the spring diet of blue tit. **B** Number of taxa within prey orders, with diverse families comprising > 10 taxa highlighted individually within their respective orders. Orders within *Insecta* (left) are split from orders within other classes (right).

4.4.3 Predictors of variation in blue tit diet

Day of year predicted a small but significant increase in dietary α-diversity over the sampled time frame, from a 4.3% chance of finding a given taxon in the diet on day 78 (19th March) to a 5.7% chance by day 128 (8th May) (Table 4.1, Figure 4.3A). Elevation and latitude did not predict any significant trend in dietary α-diversity (Table 4.1, Figures 4.3B and 4.3C). I found significant turnover in diet (β-diversity) over date, elevation and latitude (Table 4.1, Figure 4.3). However, both date and elevation predict almost twice the turnover predicted by latitude.

While none of the habitat variables predicted a significant trend in α-diversity, the coefficient for tree diversity was positive, whilst those for foliage, birch and oak were negative (Table 4.1). No significant difference was found between years. When the number of faeces in the sample is more than one there looks to be a general pattern towards having higher α-diversity, however this is not significant and seems not to differ between two and three faeces (Table 4.1).

Sites are not significantly different in the numbers of taxa present per sample (α-diversity) but show very large and significant dietary turnover (β-diversity). This is the largest source of variation found in the random effects (Table 4.1). There seems to be no residual contamination along rows or columns but there is a degree of non-independence shown within plates, indicating that there could be a level of residual contamination causing samples within plates to show some similarity in the taxa they contain (Table 4.1).

Table 4.1 Output from MCMCglmm model detailing predicted variation in blue tit diet. Estimates (coefficient) of each variable from the posterior distribution are shown alongside the 95% credible intervals (CI) and significance of fixed effect predictors (pMCMC, p ≤0.05 * ≤0.01 ** ≤0.001 ***). Interacted terms are denoted by ':'. All numeric variables are scaled to have a mean of 0 and a variance of 1. The intercept year is 2014 and number of faeces is one. Random terms are interpreted to be significant where the lower 95% CI is removed from 0.

	Coefficient	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	-3.02	-3.11	-2.93	
Year 2015	-0.018	-0.091	0.071	0.63
Date	0.051	0.020	0.081	< 0.001 ***
Latitude	0.027	-0.018	0.069	0.22
Elevation	0.0057	-0.0574	0.0699	0.88
Foliage	-0.0030	-0.0645	0.0680	0.92
Birch	-0.014	-0.057	0.033	0.55
Oak	-0.012	-0.049	0.033	0.56
Tree Diversity	0.023	-0.035	0.085	0.41
2 Faeces	0.075	-0.012	0.157	0.09
3 Faeces	0.060	-0.007	0.129	0.09
Unknown Faeces	0.018	-0.081	0.123	0.75
Random Effects				
Site	0.0030	0.0000	0.0087	
Site : Taxon	0.15	0.13	0.17	
Nestbox	0.0098	0.0036	0.0165	
Nestbox : Taxon	0.077	0.058	0.094	
Day in year : Taxon	0.00044	0.00000	0.00177	
Sample	0.026	0.019	0.033	
Sample : Taxon	0.00032	0.00000	0.00129	
Plate : Taxon	0.055	0.043	0.067	
Column : Taxon	0.00036	0.00000	0.00124	
Row : Taxon	0.00070	0.00000	0.00265	
Covariant Random Effects				
Intercept : Intercept.taxon	0.21	0.18	0.24	
Intercept : Date.taxon	0.0021	-0.0082	0.0116	
Intercept : Latitude.taxon	-0.00055	-0.00960	0.00824	
Intercept : Elevation.taxon	0.0076	-0.0022	0.0180	
Date : Date.taxon	0.025	0.018	0.032	
Date : Latitude.taxon	-0.0023	-0.0064	0.0030	
Date : Elevation.taxon	-0.0016	-0.0064	0.0019	
Latitude : Latitude.taxon	0.014	0.008	0.020	
Latitude : Elevation.taxon	0.0051	0.0009	0.0099	
Elevation : Elevation.taxon	0.026	0.019	0.035	

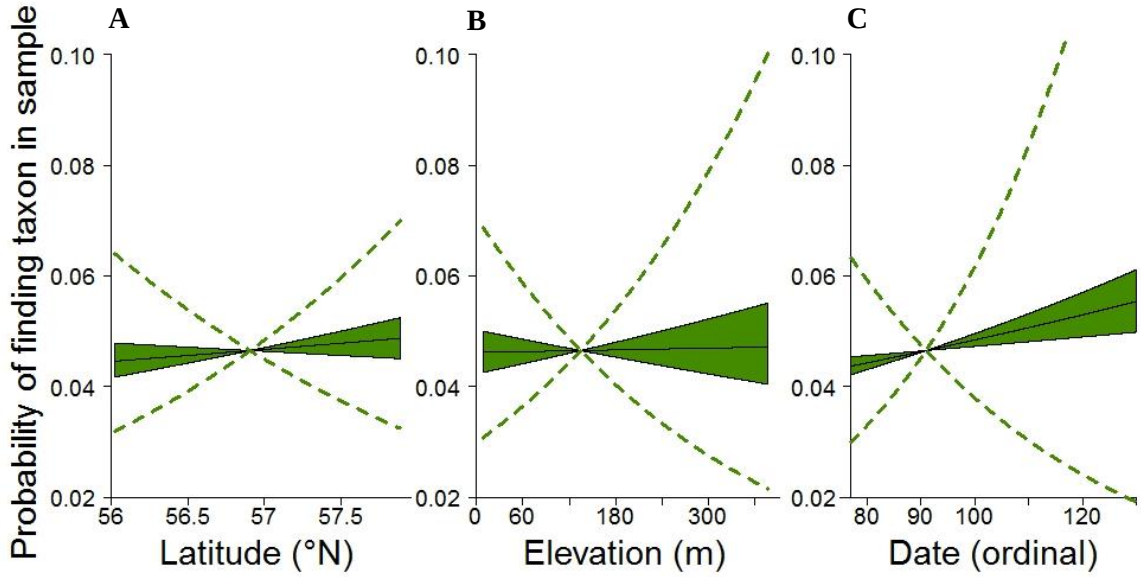

Figure 4.3 Dietary α- and β- diversity along latitudinal and elevational gradients and by date. The solid black line indicates the best model prediction of dietary α-diversity, with the green bands around it designating the 95% credible intervals in that best prediction. The green dashed line outside this specifies how β-diversity varies over the same axis, with 95% of taxa turnover responses falling within the green dashed lines. The variation in dietary species responses will follow a normal distribution around the mean within the 95% extremes plotted.

There is a significantly higher probability of finding *Diptera*, *Hemiptera*, *Hymenoptera* and *Lepidoptera* in a sample than *Araneae* and *Coleoptera*, with *Lepidoptera* having the highest probability of occurrence (Table 4.2). None of the orders studied showed any significant trend with latitude (Table 4.2, Figure 4.4A), whereas for four of the orders (*Coleoptera*, *Diptera*, *Hymenoptera* and *Lepidoptera*) probability of occurrence in a sample increased with elevation (Table 4.2, Figure 4.4B). Increasing date predicted a large and significant increase in *Hemiptera* probability, such that their occurrence exceeded that of any of the six orders at the end of the study period (Table 4.2, Figure 4.3C). Increasing tree diversity elicited a similar positive effect on *Diptera*, whereby they had the highest probability of occrrence when at the highest levels of tree diversity encountered along the transect (Table 4.2, Figure 4.3D).

Samples containing two or more faeces once again predicted, non-significantly, a higher probability of a prey item occurring, with no difference between two and three faeces per sample (Table 4.2). Year showed no significant trend (Table 4.2). The largest random effect was in taxon turnover between nestboxes within a site (Table 4.2).

Table 4.2 Output from MCMCglmm model detailing predicted probability of occurrence of six selected arthropod prey orders contributing to blue tit diet. Estimates (coefficient) of each variable from the posterior distribution are shown alongside the 95% credible intervals (CI) and significance of fixed effect predictors (pMCMC, p ≤0.05 * ≤0.01 ** ≤0.001 ***). Interacted terms are denoted by ':'. All numeric variables are scaled to have a mean of 0 and a variance of 1. The intercept year is 2014, number of faeces is one and taxon is *Araneae*.

	Coefficient	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	-0.94	-1.29	-0.63	
Year 2015	0.030	-0.219	0.245	0.79
Date	0.062	-0.086	0.234	0.46
Latitude	0.032	-0.155	0.235	0.77
Elevation	-0.19	-0.43	0.10	0.15
Foliage	0.13	-0.04	0.30	0.14
Birch	-0.049	-0.163	0.066	0.41
Oak	-0.088	-0.185	0.009	0.09
Tree Diversity	-0.058	-0.289	0.198	0.64
2 Faeces	0.15	-0.05	0.35	0.14
3 Faeces	0.13	-0.02	0.29	0.10
Unknown Faeces	-0.036	-0.260	0.214	0.77
Coleoptera	0.10	-0.24	0.44	0.56
Diptera	0.83	0.46	1.20	< 0.001 ***
Hemiptera	0.90	0.56	1.24	< 0.001 ***
Hymenoptera	0.75	0.41	1.09	< 0.001 ***
Lepidoptera	1.52	1.13	1.86	< 0.001 ***
Date : Coleoptera	0.21	-0.00	0.44	0.06
Date : Diptera	-0.015	-0.221	0.201	0.88
Date : Hemiptera	0.36	0.10	0.56	0.002 **
Date : Hymenoptera	-0.16	-0.36	0.06	0.16
Date : Lepidoptera	0.12	-0.11	0.36	0.34
Latitude : Coleoptera	0.097	-0.141	0.361	0.47
Latitude : Diptera	0.22	-0.02	0.48	0.09
Latitude : Hemiptera	-0.12	-0.41	0.12	0.36
Latitude : Hymenoptera	-0.092	-0.374	0.157	0.48
Latitude : Lepidoptera	0.20	-0.05	0.46	0.13
Elevation : Coleoptera	0.30	-0.02	0.59	0.05 *
Elevation : Diptera	0.38	0.11	0.70	0.008 **
Elevation : Hemiptera	0.24	-0.05	0.55	0.14
Elevation : Hymenoptera	0.49	0.23	0.86	< 0.001 ***
Elevation : Lepidoptera	0.51	0.20	0.79	< 0.001 ***
Tree Diversity : Coleoptera	-0.062	-0.384	0.261	0.71
Tree Diversity : Diptera	0.40	0.09	0.70	0.01 *
Tree Diversity : Hemiptera	-0.047	-0.382	0.243	0.75
Tree Diversity : Hymenoptera	-0.22	-0.56	0.08	0.18
Tree Diversity : Lepidoptera	-0.11	-0.39	0.20	0.49
Random Effects				
Site	0.021	0.000	0.066	
Site : Taxon	0.083	0.022	0.142	
Nestbox	0.036	0.000	0.075	
Nestbox : Taxon	0.19	0.12	0.28	
Day in year : Taxon	0.005	0.000	0.018	
Plate : Taxon	0.072	0.027	0.119	

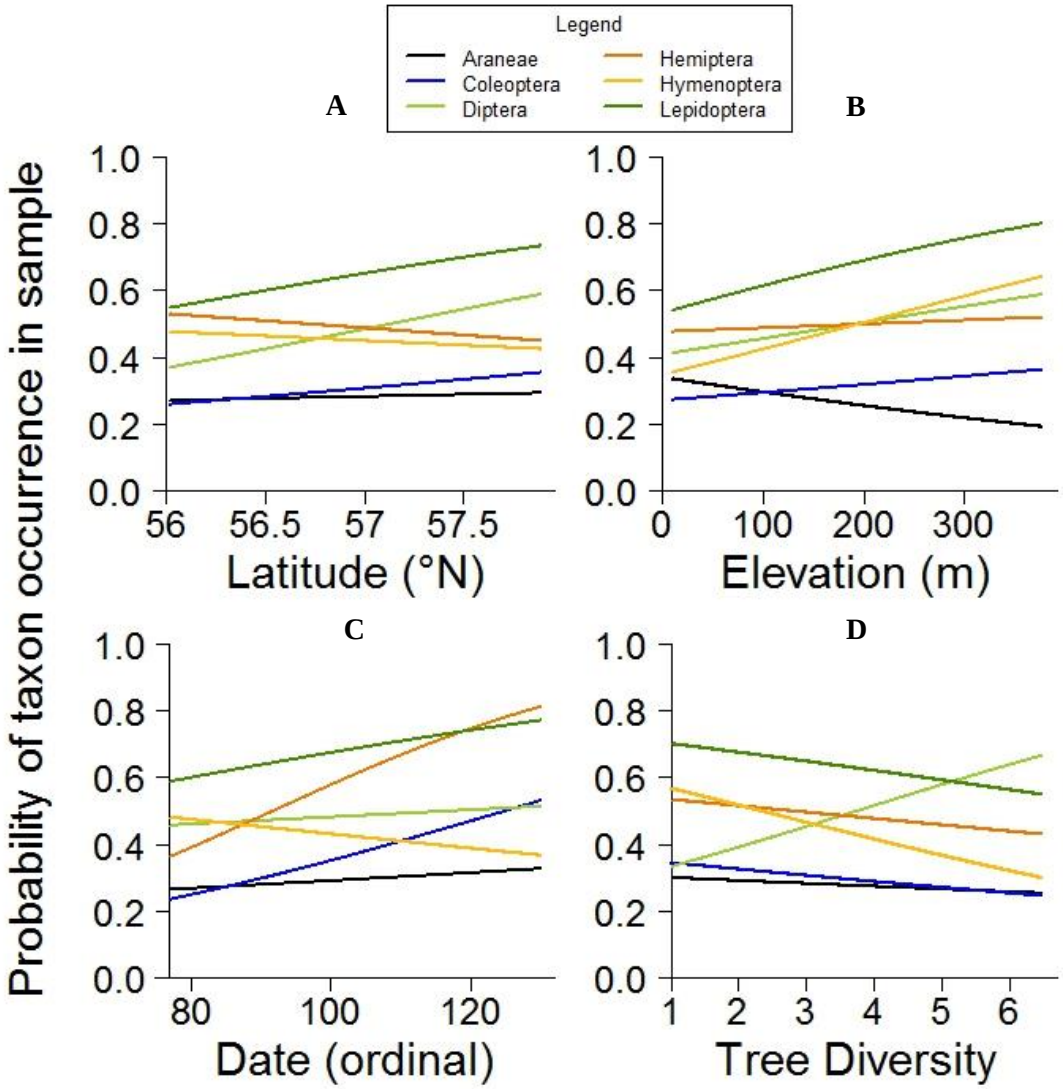

Figure 4.4 Illustrating the predictions of how each of the six arthropod prey orders examined vary in their probability of occurrence along various gradients **A** Latitude **B** Elevation **C** Date **D** Tree Diversity.

4.4.4 Dietary cue for blue tit reproduction

Significant splines, revealing a significant change in species richness per prey order over the days preceding egg laying, were found in four of the six examined arthropod prey orders, with *Araneae* and *Diptera* showing no significant trend (Table 4.3, Figures 4.5A and C). One of these trends was negative, with *Hymenoptera* showing a significant decrease in species richness in the days leading up to blue tit egg laying (Figure 4.5E). The remaining three examined prey orders (*Coleoptera*, *Hemiptera* and *Lepidoptera*) all showed a significant

increase in their species richness in samples in the days before blue tit egg laying and are thus candidates as a dietary cue used to initialise egg laying (Table 4.3). *Lepidoptera* were the most species-rich order across all dates and showed a three-fold increase in species richness within the diet in the 40 days preceding egg laying (Figure 4.5F), whilst *Hemiptera* showed a more-than four-fold increase over the same period (Figure 4.5D) and the model explained more of the variation in them than for other orders (12%, Table 4.3); *Coleoptera* showing the weakest increase (Figure 4.5B).

When presence/absence of these six prey orders is analysed instead of species richness, the same patterns emerge with the same significance (Table 4.4, Figure 4.6). However, the magnitude of the impact differs, with *Hemiptera* increasing in their chance of occurrence more than eight-fold over the 60 days preceding egg laying (Figure 4.6D), becoming the most likely dietary item present by the end of the period, and *Coleoptera* always rarer but showing a similar increase (Figure 4.6B). *Lepidoptera*, on the other hand, showed a shallower increase in presence from a higher baseline with this trend showing weaker significance than for the other two orders (Table 4.4, Figure 4.6F).

Table 4.3 Outputs from individual GAMM's predicting the number of species per selected prey order, showing estimates, test statistic, significance ($p \leq 0.05$ * ≤ 0.01 ** ≤ 0.001 ***) and adjusted r^2. Models used Poisson error structures (log link). S denotes the smooth term.

Term	Estimate/edf	t/f	p	Adjusted r^2
Araneae				0.0047
Intercept	-1.61 ± 0.17			
Year 2015	0.42 ± 0.19	2.23	0.026 *	
s	1	2.14	0.14	
Coleoptera				0.0447
Intercept	-1.12 ± 0.15			
Year 2015	-0.09 ± 0.17	-0.53	0.60	
s	3.01	8.65	< 0.001 ***	
Diptera				-0.0079
Intercept	-0.45 ± 0.15			
Year 2015	0.23 ± 0.12	1.81	0.07	
s	1	0.05	0.82	
Hempitera				0.12
Intercept	-0.71 ± 0.15			
Year 2015	0.43 ± 0.13	3.21	0.001 **	
s	1	74	< 0.001 ***	
Hymenoptera				0.025
Intercept	-0.45 ± 0.14			
Year 2015	0.02 ± 0.13	0.12	0.91	
s	4.58	5.37	< 0.001 ***	
Lepidoptera				0.075
Intercept	0.25 ± 0.10			
Year 2015	0.19 ± 0.09	2.02	0.04 *	
s	3.71	15.9	< 0.001 ***	

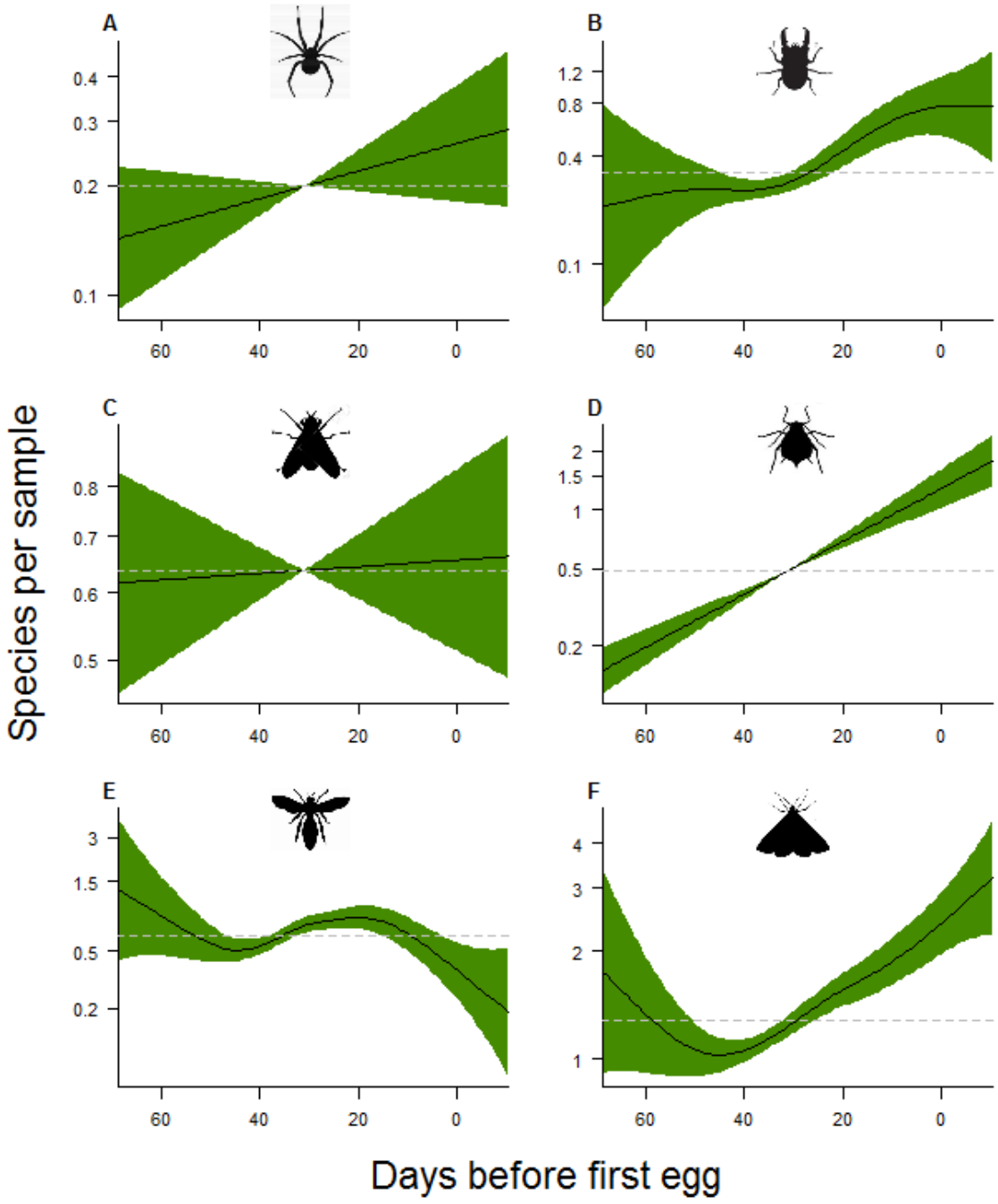

Figure 4.5 Splines showing the predicted number of species of each selected prey order across days before average first egg laying date at a site within a year. **A** *Araneae* **B** *Coleoptera* **C** *Diptera* **D** *Hemiptera* **E** *Hymenoptera* **F** *Lepidoptera*. The dashed line represents the mean number of taxa per faecal sample for that taxonomic order.

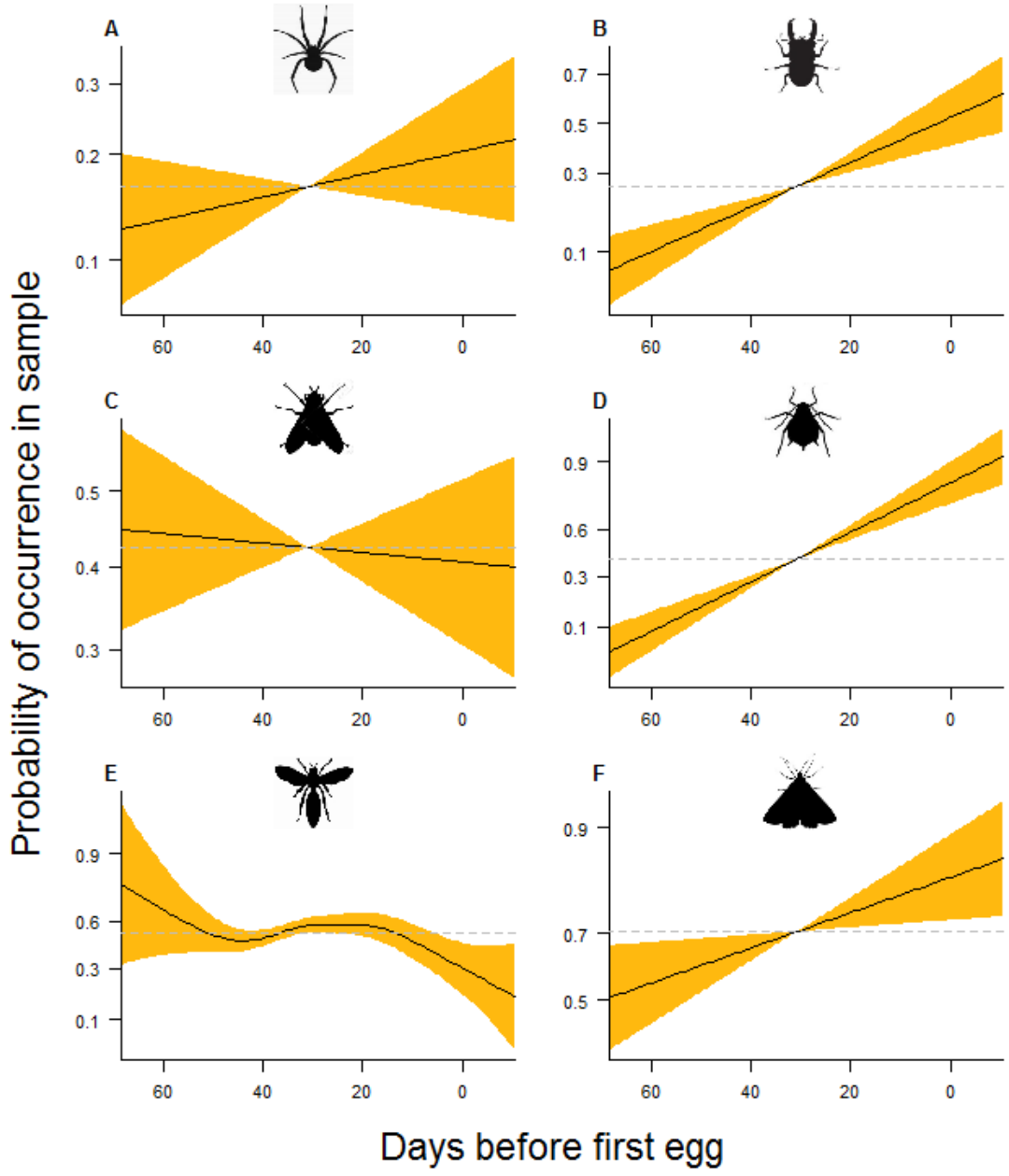

Figure 4.6 Splines showing the predicted probability of occurrence within a faecal sample of each selected prey order across days before average first egg laying date at a site within a year. **A** *Araneae* **B** *Coleoptera* **C** *Diptera* **D** *Hemiptera* **E** *Hymenoptera* **F** *Lepidoptera*. The dashed line represents the mean occurrence probability per faecal sample for that taxonomic order.

Table 4.4 Outputs from individual GAMM's predicting the presence/absence of selected prey orders, showing estimates, test statistic, significance (p ≤0.05 * ≤0.01 ** ≤0.001 ***) and adjusted r^2. All models used binomial error structures with logit link functions. S denotes the smooth term.

Term	Estimate/edf	t/f	p	Adjusted r^2
Araneae				0.0046
intercept	-1.63 ± 0.21			
year2015	0.58 ± 0.23	2.49	0.013 *	
s(d.b.fed)	1	1.28	0.26	
Coleoptera				0.031
intercept	-1.08 ± 0.21			
year2015	0.03 ± 0.24	0.13	0.89	
s(d.b.fed)	1	23.58	< 0.001 ***	
Diptera				-0.0056
intercept	-0.30 ± 0.22			
year2015	0.34 ± 0.22	1.57	0.12	
s(d.b.fed)	1	0.13	0.72	
Hempitera				0.088
intercept	-0.40 ± 0.25			
year2015	0.68 ± 0.24	2.80	0.005 **	
s(d.b.fed)	1	53.75	< 0.001 ***	
Hymenoptera				0.021
intercept	0.09 ± 0.23			
year2015	-0.20 ± 0.23	-0.88	0.38	
s(d.b.fed)	3.93	3.66	0.011 *	
Lepidoptera				0.0052
intercept	0.87 ± 0.24			
year2015	0.20 ± 0.25	0.25	0.44	
s(d.b.fed)	1	6.4	0.012 *	

4.5 Discussion

This study shows that, on the scale considered, blue tit dietary α-diversity increases as spring progresses, but is unaffected by geographic factors, whilst dietary turnover (β-diversity) is greater over temporal and elevational gradients than latitudinal. It also demonstrates how site-to-site differences in diet are large in this species at this time of year. Dietary content broadly agreed with previous work (Betts 1955; Gibb & Betts 1963) and highlighted how *Lepidoptera*, *Hemiptera* and *Coleoptera* increase in the diet pre-breeding and could provide a dietary cue to reproductive phenology, with *Hemiptera* showing the most pronounced

increase. This study also developed an operational method of metabarcoding adult bird faeces and revealed the value of controls and replicates in faecal metabarcoding studies and the necessity to incorporate them in future studies to highlight contamination events, ensure methodological accuracy and assess repeatability. Faecal metabarcoding provides excellent and highly resolved insights into faecal content and thus diet, and the repeatability of identifying specific taxa present in faecal samples is fairly high when the sample is initially split before DNA extraction, but not perfect and this possibly reflects heterogeneity within a faecal sample, and this should also be acknowledged in future studies (Jedlicka *et al.* 2017).

Blue tit dietary α-diversity increases through the spring, possibly in response to increasing environmental invertebrate diversity as spring progresses and temperatures rise (Bale *et al.* 2002; Southwood *et al.* 2004). However, this trend was not uniform in all six major prey orders, as the only significant increase over absolute time was in *Hemiptera*. When analysed in days preceding the average first lay date at a site within a year, *Lepidoptera* and *Coleoptera* also increase, with all three of these orders increasing in both species richness and likelihood of occurrence in the diet in the days before egg laying. Dietary turnover (β-diversity) was also high throughout the spring, indicating that prey items differ significantly as spring progresses, probably a result of prey phenology and variability in the temporal availability of prey items (Niemela & Haukioja 1982; Southwood *et al.* 2004). Whilst I cannot directly analyse whether a dietary cue is being utilised to control breeding phenology, as I explain in the methods, we can hypothesise that if one is being used, it could come from the overall increase in dietary α-diversity, or one of these three orders. Whilst *Lepidoptera* were common prey throughout the study period and *Coleoptera* rarer, *Hemiptera* are very rare dietary components early in the spring but increase rapidly to become the commonest by the time of egg laying and may thus be the most likely as a cue. However, *Lepidoptera*, particularly winter moth, are the primary diet of nestlings (Visser *et al.* 1998; Wilkin *et al.* 2009) and therefore would provide a more reliable cue, especially as winter moth occurred in the diet, albeit at a low occurrence. From phenological inference, the dietary winter moths found in this study were most likely to be early instar caterpillars, and therefore a highly reliable cue, as adult and pupal forms are several months removed from this time of year, whereas eggs would be more abundant earlier during the period tested and would be expected to show the opposite trend (i.e. a decline in the diet throughout the period tested), particularly as food availability is more limited earlier in the spring (see Fig C3). *Hymenoptera* in the diet decrease over this time period and *Diptera* and *Araneae* do not show a clear temporal trend.

Geographic factors, meanwhile, had no significant effect upon dietary α-diversity, but did have a large effect upon dietary β-diversity. This specifies that whilst the diversity of prey eaten may not significantly alter from place-to-place, the exact identities of these prey items do vary significantly, reinforced by site also estimating a substantial β-diversity. This could indicate local dietary specialism, but is more likely to reflect varying local prey assemblages (Southwood *et al.* 1982; Smith *et al.* 2011) and predatory opportunism. This agrees with previous faecal metabarcoding research on bats identifying the large variability of local diets (Clare *et al.* 2014a; Sedlock *et al.* 2014) and supports blue tits being generalist insectivores (Cramp & Perrins 1993). The elevation gradient in this study had similar dietary turnover to the temporal gradient, with both showing greater turnover than the latitudinal gradient, suggesting that invertebrate assemblages vary more by elevation and time than latitude over the gradients used in the context of this study, possibly due to temperature (Gaston & Williams 1996; Bale *et al.* 2002).

Although all habitat indices used in this study were non-significant predictors of blue tit dietary diversity, they do show interesting patterns. A more diverse local tree assemblage increased dietary α-diversity, whilst increased total foliage, birch and oak decreased it, albeit all non-significantly. This suggests that the range of species preyed upon increases when there is a wider diversity of local trees, probably supporting a wider range of invertebrates through the host-plant specificity exhibited by many (Southwood *et al.* 1982; Fuentes-Montemayor *et al.* 2012; Waring & Townsend 2017). Following this, the range of prey species is lowered when individual tree species become more locally abundant as they can support a less diverse invertebrate assemblage, and probably also allow the birds to hone in on certain prey species which are more locally abundant and dominant.

Blue tit prey items identified in this study broadly concur with previous studies, with the commonest six orders found also those found through physical gizzard analysis (Betts 1955; Sehhatisabet *et al.* 2008), substantiating the robustness of the faecal metabarcoding method (Zeale *et al.* 2011) and confirming core blue tit diet (Cramp & Perrins 1993). Indeed, even some of the species identified in blue tit diet in an English oak wood at this time of year were similar or identical, such as the springtail *Entomobrya nivalis*, adult *Cynipid* wasps of the *Andricus* genus, *Coleophorid* moths, and *Diptera* larvae feeding on emerging tree buds (Betts 1955). However, many of the commonest species in this study are associated with, or host specific to, birch (*Betula pubescens/pendula*), by far the commonest tree genus on this

Scottish transect (Table 2.2) and deciduous tree across Scotland (Forestry Commission 2013), and not sampled in previous dietary studies of blue tits. I find that all four of the commonest dietary *Lepiodoptera* species in this study (Figure 4.2), as well as others less common, are primarily associated (as caterpillars or pupae) with birch catkins (or under bark later in spring) at this time of year (Sterling & Parsons 2012; Waring & Townsend 2017, ukmoths.co.uk). This might explain the behavioural observations of tit species, including blue tit, feeding from catkins at this time of year (Gibb 1954; Perrins 1979; Kay 1985). In addition, blue tits seen feeding on freshly emerging aphids on birch and sycamore (Gibb 1954; Gibb & Betts 1963) were corroborated by this study as some of the commonest prey items later in spring (Figure 4.2, Table C1). The phenology of the majority of the commonest prey items means that they are only available to blue tits at this time of year, or from this time of year. These results also highlight the importance of *Lepidoptera* to adult tits, as well as nestlings (Visser *et al.* 1998; Wilkin *et al.* 2009). Most dietary items were rare, in accordance with previous faecal metabarcoding studies on generalist insectivores (Clare *et al.* 2009; Sedlock *et al.* 2014).

I have demonstrated that faecal metabarcoding can provide a robust and powerful method for assessing blue tit diet, allowing a greater sample size and taxonomic resolution than has previously been possible (Betts 1955; Sehhatisabet *et al.* 2008), as well as being non-invasive and non-destructive. I have also advanced the methodology in this study, showing the enormous value of including positive and negative controls (at both extraction and PCR stages), and sample replicates. Controls used in this study allowed the identification of contamination and the proof of positives (see also De Barba *et al.* 2014), with the positive controls in this study yielding 14 variant MOTUs for the control invertebrate, *Dryocosmus israeli*, and showing that the initial 2% similarity rule to generate independent MOTUs is likely to produce quite a few spurious taxa and highlights the value of the subsequent quality control steps. Even after strict removal of systematic contamination, some residual contamination on plates was evident and both of these issues should now be considered in future faecal metabarcoding studies. From this, I also recommend future studies to randomise PCR plate positions of samples by date, individual etc. such that any genuine and expected systematic similarity of samples is minimised.

I also demonstrate that in addition to gaining an adequate sample of faecal contents and high PCR-stage repeatability (De Barba *et al.* 2014), the ability of faecal metabarcoding to confidently assess the presence or absence of a particular taxon from a particular sample

(from separate DNA extractions), while not perfect, is fairly good, and better than a previous estimate (Jedlicka *et al.* 2017). Therefore, if the main aim of a study is to confidently identify the presence/absence of a taxon in a faecal sample I suggest that it may be worth running multiple replicates from the extraction stage onward. In addition, although the maximum number of taxa in a sample was high (n = 20), PCR competition and the methodological maximum reads per metabarcoding plate presumably places a limit on this, and reducing the number of target loci (n = 3 in this study) or samples per plate could increase the reads available per locus per sample and increase detectability. Finally, I provide a novel approach for collecting adult passerine faeces and show that despite the faeces being produced by an unseen organism and remaining in-situ for up to 48 hours pre-collection, they can still provide viable prey DNA and the species producing them can be confidently identified genetically. This method could be applied to other cavity-roosting avian species.

In summary, this study reveals the diet of a generalist passerine in finer resolution than any previous study and quantifies dietary α- and β- diversity across gradients using a novel approach. In addition I identify potential dietary cues to reproductive phenology. Blue tit dietary α-diversity increases as spring progresses, but is unaffected by geographic factors, whilst dietary turnover (β-diversity) is greater over temporal and elevational gradients than latitudinal within the parameters of this study. Site-to-site dietary variation is large. In addition, *Lepidoptera*, *Hemiptera* and *Coleoptera* all increase in the diet preceding egg laying and could possibly provide a dietary cue to reproduction, with *Hemiptera* showing the most pronounced increase. This is the largest faecal metabarcoding study to date on birds and demonstrates the potential of this technology to provide fresh insights into even well studied species.

Chapter 5

Biogeographic and host tree species effects on the spring abundance and timing of caterpillar species within temperate deciduous woodlands

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Tomatin

5.1 Abstract

The spring caterpillar biomass peak in temperate deciduous woodlands plays an important role in the productivity of many insectivorous woodland passerines, and has been invoked to understand trophic mismatch. However, the degree to which this peak varies geographically and by habitat is relatively unknown. This study aims to quantify how the likelihood of caterpillar occurrence and the temporal distribution (peak date, height and breadth) of the spring caterpillar peak vary with biogeography, tree species and the local abundance of host tree, with special attention on winter moth caterpillars as a major component. 575 caterpillars were directly collected by branch beating throughout the springs of 2014-16 from 40 woodlands along a 220km transect of Scotland across two degrees of latitude with 477 identified to species level using the COI genetic barcode. Host tree species' differed significantly in their likelihood of hosting a caterpillar, with oak and willow the most likely, and the same being true of winter moth caterpillars. Biogeography has less effect than habitat on the likelihood of caterpillar occurrence, but increasing elevation retarded peak date by 3.7 days/100m. As timings and abundances of caterpillars vary among tree species and biogeographically, this implies that the dynamics of trophic mismatch between passerine birds and the caterpillar resource may also be affected and this should be accounted for when interpreting potential mismatch in forest systems.

5.2 Introduction

Trophic mismatches, whereby consumers become temporally asynchronous with important resources and suffer deleterious effects, have received much recent research attention (Durant *et al.* 2007; Thackeray *et al.* 2016). One of the most popular study systems is the deciduous tree – caterpillar – insectivorous passerine bird food chain in temperate deciduous woodlands, principally in Europe (Visser *et al.* 1998; Both *et al.* 2006; Charmantier *et al.* 2008). At the centre of this food chain is the inter-annually variable and ephemeral annual spring caterpillar peak (Southwood *et al.* 2004; Forkner *et al.* 2008). In deciduous woodlands this peak coincides with the timing of freshly budburst leaves, before they become less palatable (Feeny 1970; van Asch & Visser 2007). Breeding in synchrony with this peak is of vital importance for the productivity of some passerine birds, such as some tit (*Paridae*) and flycatcher (*Muscicapidae*) species (Both *et al.* 2004a; Visser *et al.* 2006; Burger *et al.* 2012). Despite its central position, the caterpillar peak is the least well understood portion of this food chain, having been predominantly studied indirectly through frass fall (faecal matter) (Visser *et al.* 1998; Smith *et al.* 2011) or half fall (fully-grown caterpillars of certain species falling to earth to pupate) (Charmantier *et al.* 2008) and usually in the context of oak- (*Quercus* sp.) dominated woodlands (Charmantier *et al.* 2008; Smith *et al.* 2011). The standard methods for monitoring caterpillar biomass have limitations, with frass being only partially informative as it doesn't reveal species composition or account for frass made by other invertebrates, and half fall only capturing the full-grown larvae of certain species that descend to ground level, which may not correlate exactly with the abundance of earlier life stages of these species, or caterpillars of other species that don't descend, among the foliage.

Temperate deciduous woodlands comprise many different tree species across wide latitudinal and elevational gradients and the passerine birds studied, such as tits, are often woodland generalists (Perrins 1979; Blair & Hagemeijer 1997). Therefore, it is important to understand how the caterpillar peak varies across biogeographical gradients and on tree species other than oak to create a clearer picture of how phenological mismatch is acting in the landscape as a whole (Both *et al.* 2004b; Burger *et al.* 2012). Various aspects of the caterpillar peak could vary, including the height (peak biomass), the timing of the peak date and the breadth (duration) of the peak (Figure 5.1A). It is possible that in regions or years when mismatch between the timing of peak avian demand and the timing of the oak caterpillar peak is pronounced, deleterious effects on local avian productivity could be buffered locally by differing caterpillar peaks on other tree species providing alternative

resources (Figure 5.1B). It is also possible that any consequent negative effect on avian demography from local mismatch could be buffered at a landscape level by matched caterpillar peaks at other locations (Figure 5.1D). For instance, in schematic Figure 5.1D location C could buffer birds breeding too late in the year to synchronise with the caterpillar peak at locations A and B, but if all locations were like A and B within a landscape; no buffering would be possible (assuming passerine breeding time and peak demand to be similar across locations).

In temperate deciduous woodlands the spring caterpillar peak is often dominated by one or two abundant species (Hunter 1992; Butler & Strazanac 2000; Wesolowski & Rowinski 2006a). Among European studies on woodland caterpillars, winter moth (*Operophtera brumata*) is the most commonly mentioned abundant species, especially among oaks. Winter moth is an abundant generalist feeding on many tree species (Kerslake & Hartley 1997; Wesolowski & Rowinski 2006a; Waring & Townsend 2017) and an important component of woodland passerine diet, especially for nestlings (Visser *et al.* 1998; Wilkin *et al.* 2009; Cholewa & Wesołowski 2011). In winter moths, synchrony of larval development with host leaf bud burst has been shown to be important for growth and survival (Feeny 1970; Buse & Good 1996), as in many other spring-feeding caterpillar species (Klemola *et al.* 2003; van Asch & Visser 2007). Leafing phenology of deciduous tree species responds to temperature (Polgar & Primack 2011; Roberts *et al.* 2015) and winter moths seem able to synchronise well with host plants by responding to similar temperature cues (Buse & Good 1996; Buse *et al.* 1999; van Asch *et al.* 2012), as do some other studied spring caterpillar species, such as the autumnal moth (*Epirrita autumnata*) (Klemola *et al.* 2003). In years with high synchrony, outbreaks may occur (van Asch & Visser 2007; Donaldson & Lindroth 2008), and it has been suggested that such outbreaks may be more common at higher elevations in sitka spruce (*Picea sitchensis*) and heather (*Calluna vulgaris*) than in lowland oak, due to a dearth of controlling natural enemies in the former (Raymond *et al.* 2002). Other locally abundant caterpillar species, such as green oak tortrix (*Tortrix viridana*), may also be important to the birds, and together these two caterpillar species were estimated to comprise c.75% of the spring caterpillar peak in southern England (Hunter 1990, 1992), with winter moth alone responsible for over 80% of the peak in a primeval Polish forest during an outbreak year (Wesolowski & Rowinski 2006a), illustrating the dominance of certain species. Thus, certain caterpillar species may contribute more to a higher spring peak (Figure 5.1C) and be of more import to avian consumers.

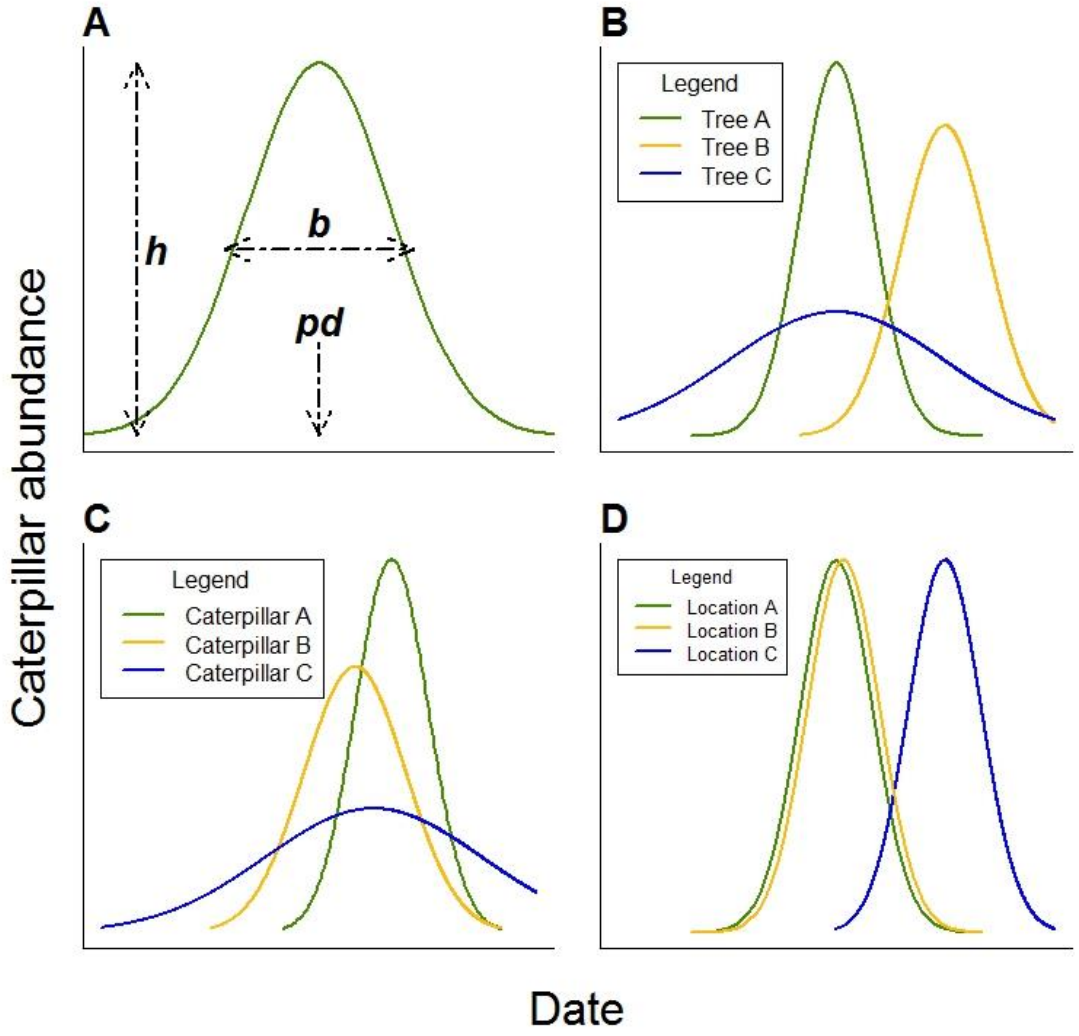

Figure 5.1 Schematic diagrams illustrating potential caterpillar biomass spring peaks. Plot **A** shows the parameters of caterpillar temporal distribution that could vary; ***pd*** the peak date ***h*** the height of the peak and ***b*** the breadth (duration) of the peak (50% of total peak). Plots B-C present different contributions to variation in caterpillar temporal distributions. **B** shows how different tree taxa may have different caterpillar temporal distributions, with tree B showing a later peak than trees A and C and tree C having a lower, longer peak than trees A and B. **C** illustrates how different caterpillar species may show different spring peak even on the same tree, with caterpillar A having the highest peak and caterpillar C the longest. **D** illustrates how geographical locations could have differently timed spring caterpillar peaks, with locations A and B sharing a similar peak date whilst location C has a later peak date.

Environmental variables that co-vary with elevation and latitude, such as the thermal environment, may govern the temporal availability and abundance of caterpillars as caterpillar development is known to slow considerably in colder environments (Buse *et al.* 1999; Milonas & Savopoulou-Soultani 2000), with many invertebrate species' distributions thermally limited (Bale *et al.* 2002). This is manifested in a tendency for lower total

invertebrate abundance and diversity at higher elevations (Garibaldi, Kitzberger & Chaneton 2011; Pellissier *et al.* 2012) and lower body mass and survival of caterpillars at the higher ranges of their elevational distributions (Alonso 1999). This may suggest that increasing elevation will reduce the height of the caterpillar biomass peak. Alternatively, predation of caterpillars is higher at lower elevations and latitudes (Roslin *et al.* 2017) and this has been implicated as the factor contributing to the pattern of more outbreaks of winter moth caterpillars at higher elevations (Raymond *et al.* 2002), which could indicate the opposite tendency (higher caterpillar abundance at higher elevations). In addition, increasing elevation could delay the timing of the peak as other invertebrates, such as the spittlebug *Neophilaenus lineatus*, have been observed emerging up to four weeks later along a 440m altitudinal gradient in the UK (Fielding *et al.* 1999). Timing of the peak could also be affected by latitude, with more northerly latitudes across the UK showing later peaks, with the duration also lasting longer (Smith *et al.* 2011). However, at a smaller scale (spanning 4.5° latitude), latitude had no effect on spittlebug emergence timing (Fielding *et al.* 1999). All of these factors could impact the temporal distribution of spring caterpillar peaks across elevational and latitudinal gradients and result in geographical variation in the caterpillar peak (see Figure 5.1D).

Host-tree specificity among woodland caterpillars is high and certain tree species host higher caterpillar diversity than others (Kennedy & Southwood 1984; Summerville *et al.* 2003; Sterling & Parsons 2012; Waring & Townsend 2017). Diversity is higher on native (Southwood *et al.* 1982; Burghardt, Tallamy & Shriver 2009; Fuentes-Montemayor *et al.* 2012), and more locally abundant tree species (Niemela *et al.* 1982; Southwood *et al.* 1982; Kelly & Southwood 1999) and higher local tree species diversity increases total levels of local caterpillar diversity (Fuentes-Montemayor *et al.* 2012; Stireman III, Devlin & Doyle 2014). In the UK, oak and willow (*Salix* sp.) harbour the highest diversities of caterpillar species, followed by birch (*Betula* sp.) with the other tree species supporting varying lower species richness (Kennedy & Southwood 1984).

Host-tree species also influences the abundance of caterpillars found, with tree species varying in the average total caterpillar biomass they support (Butler & Strazanac 2000; Marshall & Cooper 2004; Veen *et al.* 2010). It is unknown if tree species hosting higher caterpillar diversities also host higher abundances. However, higher local tree species diversity increases the abundance of caterpillars (Southwood *et al.* 1982; Stireman III *et al.* 2014), as do larger contiguous woodlands with greater connectivity (Marciniak *et al.* 2007;

Stireman III *et al.* 2014). Winter moths, for example, are less common and sometimes absent in small and fragmented stands (van Dongen *et al.* 1994) and are more likely to outbreak in larger woodland blocks (Wesolowski & Rowinski 2006a). Even within generalist feeders, like winter moth, abundances can vary between different host trees. This could be due to certain host trees boosting growth rates (Schwartzberg *et al.* 2014) or final caterpillar weight (Singer *et al.* 2012) through being more nutritious. Host plant use of generalist feeders can also vary due to elevation (Bale *et al.* 2002) or host tree-specific parasitoids (Lill, Marquis & Ricklefs 2002). Winter moth caterpillars also grow better on their original host plant species than when translocated onto novel, but still edible, hosts (Kerslake & Hartley 1997). Even tree structure and woodland management can affect caterpillar abundances, with different assemblages and densities of different species between the understory and canopy, possibly caused by tannin or pubescence variations, both of which reduce the palatability of leaves (Feeny 1970; Forkner *et al.* 2004; Lill *et al.* 2006).

It is possible that winter moths and other generalist feeders may be locally adapted to be synchronous with the phenology of their most important/common host plant in a particular landscape and opportunistically feed on other hosts. This seems to be the case in a Polish primeval forest, where hornbeam (*Carpinus betulus*) was the commonest local tree and the most heavily affected by winter moth outbreak, hosting the highest abundances. More or less concurrently budbursting tree species were also affected with all other deciduous trees affected to a much lesser extent (Wesolowski & Rowinski 2006a). This highlights the possibility of a synchronous winter moth peak across tree species centred to coincide with the budburst of the commonest/most productive local tree. This would lead to synchronous caterpillar peaks across tree species and reduce the potential for phenological buffering of trophic mismatch (Figure 5.1B). Counter to this, winter moths can synchronise their phenology very locally, even to individual trees (van Dongen *et al.* 1997; Hinks *et al.* 2015), with earlier budbursting oak trees sustaining higher caterpillar abundances (Hunter 1992), and this may extend the duration of the food peak at a local scale, buffering trophic mismatch.

The timing of the caterpillar peak is also thought to vary among tree species, with tree species that have one new leaf growth per year (e.g. oak) supporting higher diversities early in the spring, whilst those with multiple new leaf growths per year (e.g. aspen (*Populus tremula/tremuloides*)) supporting higher diversities later in the summer (Niemela & Haukioja 1982). However, among the native constituent species of a single genus (e.g. oak)

in the UK, the temporal distribution of the caterpillar peak appears to be very similar (Southwood *et al.* 2004). A study in Sweden found the temporal distributions of caterpillar peaks of four deciduous tree genera to be broadly similar and earlier, narrower and much higher than those of two coniferous genera (Veen *et al.* 2010). This seems to be supported by diet data in pied flycatchers (*Ficedula hypoleuca*) showing that caterpillar content is higher in deciduous woodlands than coniferous, and shows a greater seasonal decline in the former (Burger *et al.* 2012). However, even within the deciduous genera studied by Veen *et al* 2010, the temporal distribution of caterpillars differed, with the oak peak being higher and the birch peak slightly earlier.

Among site differences in local caterpillar assemblages, diversity and abundance caused by biogeographical, habitat and host tree species variation could generate substantial differences in the temporal distribution of the spring caterpillar peak. This in turn could have consequences for insectivorous birds and give rise to geographic variation in how trophic mismatch operates in this system (Both *et al.* 2004b; Burger *et al.* 2012). Therefore, the aim of this study is to identify the effects of tree species, habitat and biogeographic variables on the temporal distribution of caterpillars (see Figure 5.1). First, I quantify the species composition of the spring caterpillar community and estimate the contribution made by winter moths. Next I estimate the effects of tree species, habitat, latitude and elevation on the presence (as a proxy for abundance) of both caterpillars in general and winter moths in particular. Lastly, I estimate the effects of elevation and tree species on the timing, breadth and height of the caterpillar availability and biomass peak and try to discern whether the relationship between tree phenology and caterpillar peak is similar across tree species, with a view to better illuminating how the spring caterpillar peak may vary at a landscape level and whether it is synchronous or differs among tree species. Throughout I use direct sampling of caterpillars from foliage, which are identified to species level genetically, as it is difficult to reliably visually distinguish caterpillars to species level. In the future this work will allow for comparison of the environmental availability of caterpillar species with the dietary composition and fitness of insectivorous passerines to ascertain which constituent species of the peak are of greater importance in trophic mismatch.

5.3 Methods

5.3.1 Study system

This study was conducted along a 220 km transect of Scotland incorporating 40 woodland field sites, detailed in 2.3.1. All dates used in this study, unless explicitly indicated otherwise, are ordinal dates counted from January 1st, meaning that April 1st is day 91 in most years and day 92 in a leap year. The location of each nestbox was determined using a handheld GPS (Garmin eTrex High Sensitivity) and I obtained elevation (meters above sea level (m.a.s.l)) via the Google Maps elevation API. Habitat surveys were conducted at each of the 40 field sites as detailed in 2.3.2 and spring tree phenology (first budburst, complete budburst, first leaf, complete leaf) was studied on 6-10 individually identifiable and labelled focal trees per intensively studied site in each year as detailed in 3.3.3. Each focal tree was assigned a unique identifier and identified to genus level (summarised in Tables 2.2 and 3.1), as were all of the trees included in the habitat survey (2.3.2).

5.3.2 Caterpillar sampling

Caterpillar sampling was conducted via branch beating, starting at all intensively studied sites (Table 2.1) the day immediately after the day when a threshold of 45% of focal trees along the entire transect were at, or beyond, the first leaf stage of their phenology in that given year, and continuing until the end of the field season in each year. In 2014 these sampling dates were days 120 - 166, in 2015 days 125 - 175 and in 2016 days 130 - 173. The aim was to start sampling phytophagous invertebrates as early in the year as possible, whilst minimising damage to underdeveloped buds and leaves on focal trees. Branch beating trees were selected at random from the pool of focal trees at each site (see 3.3.3 and Table 3.1), subject to the constraint that the tree had at least one branch with a minimum length of 1m between 0.5-1.5m above the floor. Suitable branches on selected focal trees were given numbers and one randomly selected for beating. Branches were identified with string and maintained as the beaten branch for that focal tree, except in a limited number of cases where the branch broke or died. In 2014, two focal trees were beaten on the first visit and a different focal tree the next visit (two days later), returning to the first two the visit after and continuing in that pattern until the conclusion of the field season in that year, such that each branch was beaten every four days. In 2015 and 2016 the sampled branches were increased to four branches on the first visit and two on the second.

Beating was into a clear plastic rubble sack (76 x 51 cm) at its full extent over as much of the branch and foliage as possible, holding the open end closed and facing it upwards, and then beating the bag with a hand 30 times at regular intervals and strength (about 2 per second) to dislodge invertebrates on the branch into the bag. After 30 beats, everything within the bag was counted. All caterpillars (invertebrate larvae appearing like those of *Lepidoptera* sp.) with an estimated diameter ≥ 1mm were counted and collected by the beater. Collected caterpillars were stored in pure ethanol filled, individually labelled Eppendorf tubes and placed in a freezer. The weather in three categories (dry/wet/rain) and identity of the beater was recorded for each beating.

5.3.3 Caterpillar identification

For all stored caterpillars from 2014-16 I measured the maximum length and width (mm), with the exclusion of samples that had become desiccated and thus no longer resembled their original proportions. A small portion of each (e.g. prolegs) was removed with a sterilised scalpel. PCR and barcoding of 380 non-desiccated samples at the cytochrome c oxidase subunit I (CoI-5P) locus (640 BP) was conducted by the Biodiversity of Life Database (BOLD) in Guelph, Canada (Ratnasingham & Hebert 2007). Each was photographed and all can be accessed through the BOLD project BLUTI (sequence pages BLUTI001-380, www.boldsystems.org). 317 samples were fully barcode compliant by having two complete sequences (forward and reverse) and a further 44 had one complete sequence. All 361 of these samples were queried against BOLD and GenBank databases, with the best hit accepted, all to species level. Of the 19 samples that failed to record a full sequence, 11 were assigned to species level based on the following: the best hit had > 85% identity match identical including unread bases ("N") and > 98% when unread bases were ignored, the species was already known to occur on the transect through a successful barcode, and the best hit species was > 2% better than the next best hit species. Incomplete barcodes that did not meet these criteria were recorded as unidentified (n = 8). Where > 1 visually identical caterpillar was collected from the same beat, one was sent to BOLD and the other(s) assumed to be of the same taxon, with this recorded as visually rather than genetically identified (105 samples visually identified).

5.3.4 Statistical analyses

Caterpillar number per beating was converted into presence/absence for analysis. Whilst this meant discarding some information, I had insufficient cases where n > 1 to use this information. To assess whether caterpillars are more frequently found where their host food plant is more abundant I calculated local tree resource availability as the percentage of trees at the site that are of the same genus as the beaten tree.

I used a Bayesian generalised linear mixed model (GLMM) in the MCMCglmm package (Hadfield 2010) to analyse how the probability of finding a caterpillar via branch beating varies across biogeography, habitat and host tree species. Caterpillar presence/absence in a beating sample was the response variable, with site mean latitude (°), site mean elevation (m), year and local tree availability as fixed effects and tree species beaten, site, individual tree ID, date within year and recorder ID as random effects, with a categorical error structure (logit link function). Posterior distributions for the best linear unbiased predictions (BLUPs) were retained. I did not aim to simplify this model and significance was judged on the basis of credible intervals.

A separate, otherwise identical GLMM, was conducted only containing presence/absence of winter moth caterpillars as the response variable to assess the factors underpinning the likelihood of finding this, the most common, widespread and cosmopolitan caterpillar found along the transect.

To assess the effect of elevation on the temporal distribution of the caterpillar peak – omitting any effect of tree species – I used a GLMM with caterpillar presence/absence in a beating sample as the response variable; date, date^2, elevation and year with interactions between elevation and date and elevation and date^2 included as fixed effects and site and tree ID as random effects, with a categorical error structure. I obtained elevation specific predictions of peak date (the date on which the likelihood of finding a caterpillar was predicted to be at its highest), height of peak (the probability of finding a caterpillar on the peak date) and breadth of peak (length of time either side of peak date where the probability of finding a caterpillar is 50% of the probability at the height of the peak or more) from the posterior distributions of the model. A nominate 50% peak was used to denote peak breadth as this was felt to provide a large food supply to predatory birds and as the peaks are

symmetrical, breadths would be linearly related and comparisons between peaks identical regardless of the cut-off chosen (see section D1).

To assess how tree species differ in the length of time after they bud burst until reaching a caterpillar peak and how they affect the temporal distribution and shape of the caterpillar peak, the dataset was reduced to beatings from four tree species each of which had >50 caterpillars collected from them: Birch, Oak, Sycamore and Willow. For each individual tree in each individual year the time since first bud burst (FBB) for each sampling date was calculated. I used a GLMM with presence/absence of caterpillars as the response and time since FBB, time since FBB^2, tree species and year with interactions between tree species and time since FBB and FBB^2 fitted as fixed effects. The random effects were site and tree ID.

To project the temporal trends in caterpillar temporal distributions on the four tree species in terms of ordinal date rather than time after FBB, I considered a single site (STY, mean 56.48°N, -3.47°E, see Table 2.1), which was the only site along the transect with at least one FBB date recorded for each of the four focal tree species in every year. The mean FBB of each tree species across years was calculated and this date added to time since FBB to derive a prediction of the caterpillar temporal distribution across ordinal dates.

To test whether the temporal distribution of caterpillar biomass departs from the temporal distribution of caterpillar presence/absence I reduced the dataset further (within the four best estimated tree species as above) to only include successful beatings with measured caterpillars and an estimated volume (termed biomass for this study) of each caterpillar was calculated on the basis of πr^2, where r = radius. A GLMM similar to the one detailed above for assessing the presence/absence of caterpillars was then used to analyse this, with the response being log-transformed caterpillar biomass rather than presence/absence, and a Gaussian error structure rather than categorical. I then calculated predicted values for biomass of caterpillars on specific tree species across days since FBB by multiplying the posterior distribution of predicted values from the biomass and presence/absence models.

All GLMMs were run with sufficient iterations such that effective sample sizes for all the focal parameters exceeded a minimum of 200 and autocorrelation was ensured to be low. Numeric predictor variables, including dates and timings, were mean-centred for all models for ease of modelling and interpretation (Schielzeth 2010). Parameter expanded priors were used for all models, with fixed residual variance for categorical error structure models. A

Bayesian equivalent of a p value is calculated by determining what proportion of the posterior distribution is less than or greater than 0, taking the smaller of these and multiplying by two for a two-tailed p value. All analyses were conducted in R version 3.1.1 (R Core Team 2014).

5.4 Results

5.4.1 Summary of caterpillars sampled

A total of 575 caterpillars were collected over the course of the study and 477 identified to species level (see 5.3.3), comprising 62 species. Some larvae collected were not *Lepidoptera*, but were visually similar, and included for analyses as they contribute to insectivorous bird diet and have been retained by some previous studies (Betts 1955; Marciniak *et al.* 2007). The 477 identified caterpillars included 445 *Lepidoptera* larvae (93.3%) of 45 species, 15 *Hymenoptera* larvae (3.1%) of 13 species, 11 *Diptera* larvae (2.3%) of 3 species and 6 *Coleoptera* larvae (1.3%) of 1 species. Within the *Lepidoptera*, the most important constituent families were the *Geometrids* (347 individuals (78% of *Lepidoptera*) of 21 species) and the *Noctuids* (56 individuals (13% of *Lepidoptera*) of 10 species). Most species were rarely sampled, with only eight species comprising 15 or more identified individuals (winter moth *Operophtera brumata* 156, scarce umber *Agriopis aurantiaria* 67, northern winter moth *Operophtera fagata* 27, variable smudge *Ypsolopha ustella* 19, mottled umber *Erannis defoliaria* 17, dotted border *Agriopis marginaria* 16, common quaker *Orthosia cerasi* 16, the chestnut *Conistra vaccinii* 15) and the three most commonly sampled species comprising over 50% of the total caterpillars identified (Figure 5.2). See Table D1 for a complete list of species found and the total numbers and host tree species of each, along with sites they were collected at.

There is clear evidence that species richness varies among tree species, being highest on oak from direct sampling (Figure D6), but estimated to be highest on willow from Chao estimated species richness pools (Figure 5.2 inset). Estimated species richness via a species accumulation curve for the whole transect is shown in Figure D7. Latitudinal and elevational trends in the presence and abundance of the eight most commonly sampled species can be seen in Figures D1 and D2. It can be seen from these figures and Table D1 that winter moth appears to occur almost everywhere whilst scarce umber and northern winter moth favour birch-dominated sites, scarce umber being more numerous at higher elevations, and the

chestnut only occurring at lower elevations along the transect. Figure D3 shows species richness per site, which ranges from one to eleven.

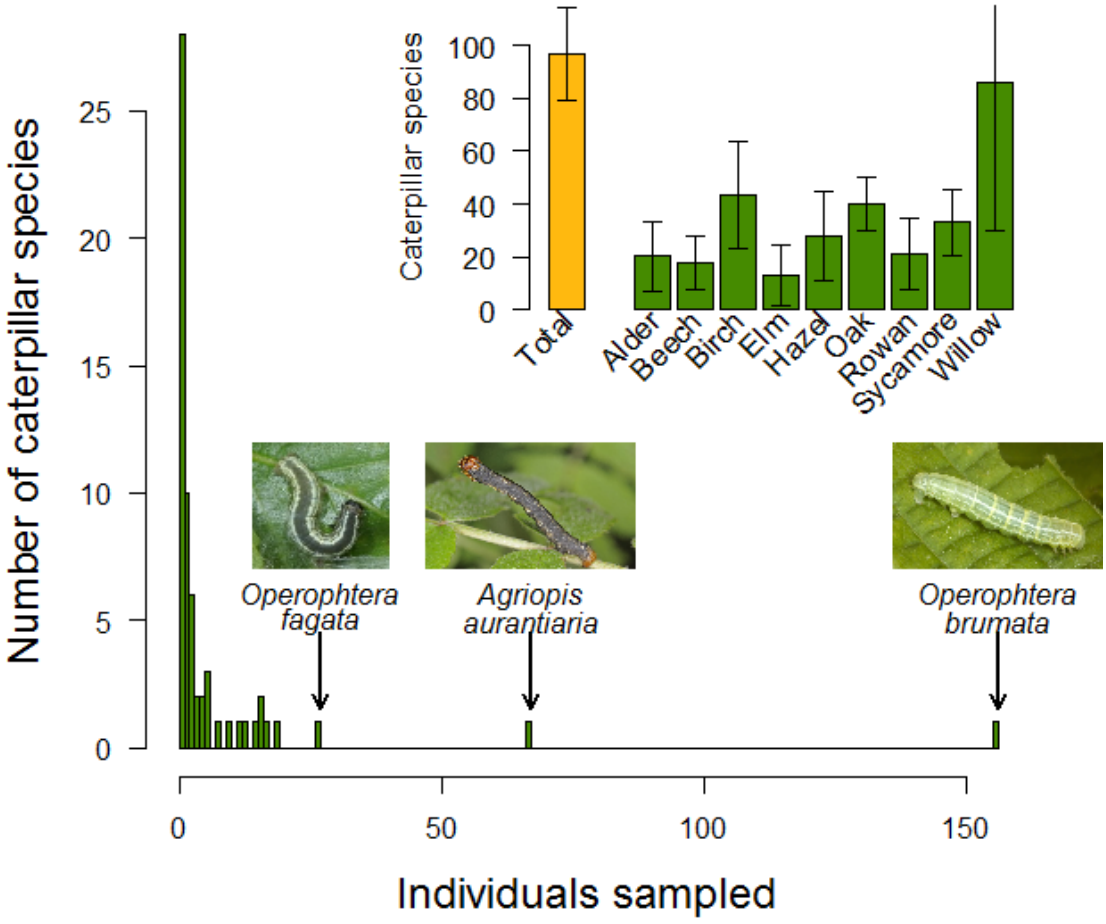

Figure 5.2 Histogram of the number of individuals sampled of each species, with pictures of the three most abundant species. **Inset** Estimated caterpillar species richness accounting for sampling effort associated with tree species' in this study, along with the estimated caterpillar species richness of the transect as a whole (total). Species richness was estimated in the R package vegan (Oksanen *et al.* 2012) which implements the Chao equation, with Chao estimated species pools ± se displayed for each tree species and the total transect. Tree species branch beaten but not included in this inset figure (Ash, Aspen, Cherry, Chestnut and Lime) yielded fewer than five caterpillar species and therefore their species richness pool could not be estimated. The actual number of caterpillar species sampled for each tree species is shown in Fig D6. Tree taxa detailed are those shown in Tables 2.2 and 3.1, where species within each taxon are described.

5.4.2 Caterpillar presence across biogeography, habitat and host tree species

The probability of sampling a caterpillar showed no significant latitudinal or elevational trends, nor was there a significant effect of the amount of host tree species locally available (Table 5.1). Caterpillars were sampled at a significantly higher rate in 2014 than in 2015 or 2016. The probability of sampling a caterpillar varied significantly among tree species and the variation among dates within a year is of similar magnitude. Among site variation and the effect of individual tree ID is much less pronounced and recorder ID appears poorly estimated.

The 95% credible intervals derived from the BLUPs for tree species reveal a significantly greater probability of sampling a caterpillar on oak and willow than the other recorded tree species (Figure 5.3). While the BLUPs for the other species do not deviate significantly from 0, the median BLUP for birch is positive, whereas for alder and ash it is negative.

Table 5.1 Biogeographical, year and habitat predictors of the probability of sampling a caterpillar, together with 95% credible intervals (CI), estimated from a GLMM (see 5.3.4).

	Coefficient	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept (2014)	-3.70	-6.58	-1.59	
Latitude	0.015	-0.475	0.505	0.96
Elevation	0.00015	-0.00221	0.00241	0.90
Year 2015	-1.72	-2.32	-1.12	< 0.001 ***
Year 2016	-1.05	-1.63	-0.46	< 0.001 ***
Habitat Availability	0.0039	-0.0024	0.0103	0.24
Random Effects				
Tree Species	0.94	0.11	2.37	
Site	0.36	0.12	0.66	
Tree ID	0.22	0.00	0.43	
Date within year	1.15	0.63	1.67	
Recorder ID	7.02	0.00	34.43	

5.4.3 Winter moth presence across biogeography, habitat and host tree species

For winter moths the inter-annual trend is weaker and non-significant, and there remains no trend in the probability of occurrence with latitude or elevation (Table 5.2). However, the availability of host tree species significantly predicts occurrence, with rarer tree species in the local environment having a greater probability of sampling a winter moth caterpillar. The probability of sampling a winter moth varied significantly among dates within a year, with variance between tree species of a similar magnitude but with a broader posterior distribution (i.e. greater uncertainty). Variance among sites was slightly less. The effect of individual tree ID is much less pronounced and recorder ID was again poorly estimated.

Oak and willow had a greater than average probability of hosting a winter moth caterpillar, which is similar to the effects estimated for all caterpillars (Figure 5.3). None of the BLUPs for other tree species deviated significantly from the average probability, though rowan and alder were the least likely to host a winter moth caterpillar at the time of year tested.

Table 5.2 Biogeographical, year and habitat predictors of the probability of sampling a winter moth caterpillar, together with 95% credible intervals (CI), estimated from a GLMM (see 5.3.4).

	Coefficient	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	-5.53	-7.40	-3.72	
Latitude	-0.21	-0.99	0.64	0.61
Elevation	0.0020	-0.0019	0.0060	0.30
Year 2015	-0.90	-1.92	0.13	0.09
Year 2016	0.063	-0.91	1.11	0.92
Habitat Availability	-0.013	-0.024	-0.001	0.03 *
Random Effects				
Tree Species	1.32	0.05	3.50	
Site	0.81	0.11	1.64	
Tree ID	0.33	0.00	0.91	
Date within year	1.68	0.65	2.90	
Recorder ID	2.35	0.00	8.99	

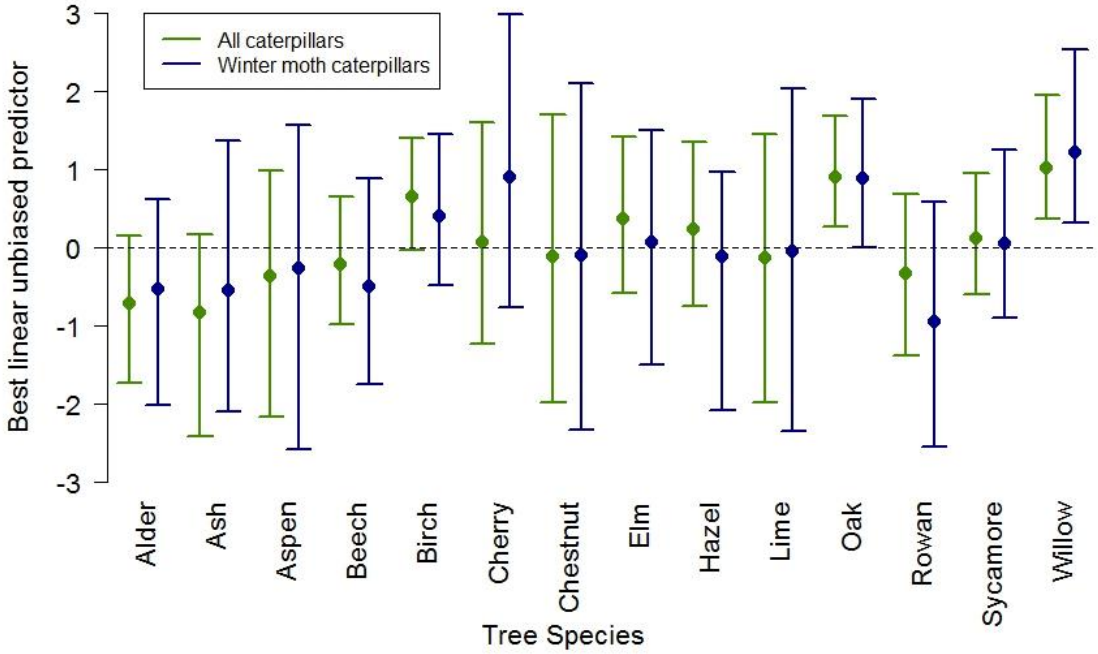

Figure 5.3 The posterior median and 95% credible intervals of the BLUPs for each tree species when analysed as a random effect in the models described in Tables 5.2 (all caterpillars, green) and 5.3 (winter moth caterpillars, blue). Credible intervals that do not cross 0 correspond to BLUPS that depart significantly from the mean effect.

5.4.4 Elevational effects on the temporal distribution of the spring caterpillar peak

When the effects of tree species are excluded the effect of elevation on the temporal distribution of the spring caterpillar peak is pronounced (Table 5.3). A later date correlated with a significantly higher likelihood of sampling a caterpillar, but the quadratic date term was negative, implying a humped relationship (Table 5.3). In addition, there was a significant interaction between date and elevation such that increasing elevation delayed the peak date of caterpillars. These effects mean that the caterpillar peak at 450 metres above sea level (m.a.s.l) is predicted to be both later in the year and higher than the peak at sea level (Figure 5.4). The timing of the caterpillar peak was estimated to be 16.7 days earlier at sea level than at 450 m.a.s.l (95% credible interval = -36.9 - -4.5), whilst the peak probability of sampling a caterpillar was also significantly lower at sea level than 450 m.a.s.l (-0.31, 95% credible interval = -0.60 - -0.05) (Figure 5.5). The breadth of the peak was not significantly different (95% credible interval = -34.7 – 17.3, median -2.5). The predictions from sea level are more tightly estimated (Figure 5.5).

Table 5.3 Elevation and timing predictors of the probability of finding a caterpillar via branch beating, together with 95% credible intervals (CI), estimated from a GLMM (see 5.3.4).

	Slope	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	-1.67	-2.00	-1.32	
Date	0.072	0.060	0.085	< 0.001 ***
Date^2	-0.0032	-0.0041	-0.0024	< 0.001 ***
Elevation	-0.000062	-0.0025	0.0024	0.95
Year 2015	-1.86	-2.18	-1.54	< 0.001 ***
Year 2016	-1.39	-1.70	-1.08	< 0.001 ***
Date : Elevation	0.00026	0.00015	0.00038	< 0.001 ***
Date^2 : Elevation	-0.00000097	-0.00000792	0.00000599	0.74
Random Effects				
Site	0.50	0.19	0.86	
Tree ID	0.39	0.13	0.67	

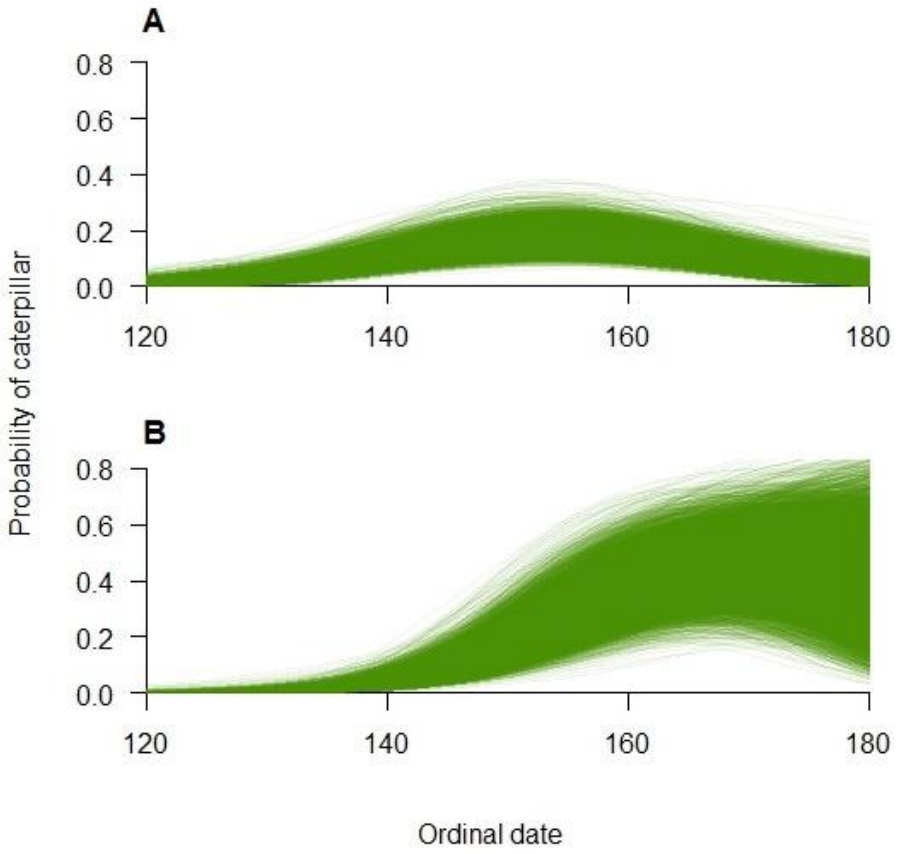

Figure 5.4 Predicted probability of finding a caterpillar via branch beating throughout spring (ordinal date) **A** at sea level **B** at 450 m.a.s.l (elevations roughly equivalent to the lowest and highest on the transect (Figure 2.1B, Table 2.1)). Posterior distributions from GLMM reported in Table 5.3 for 2014 and with all other variables at their mean.

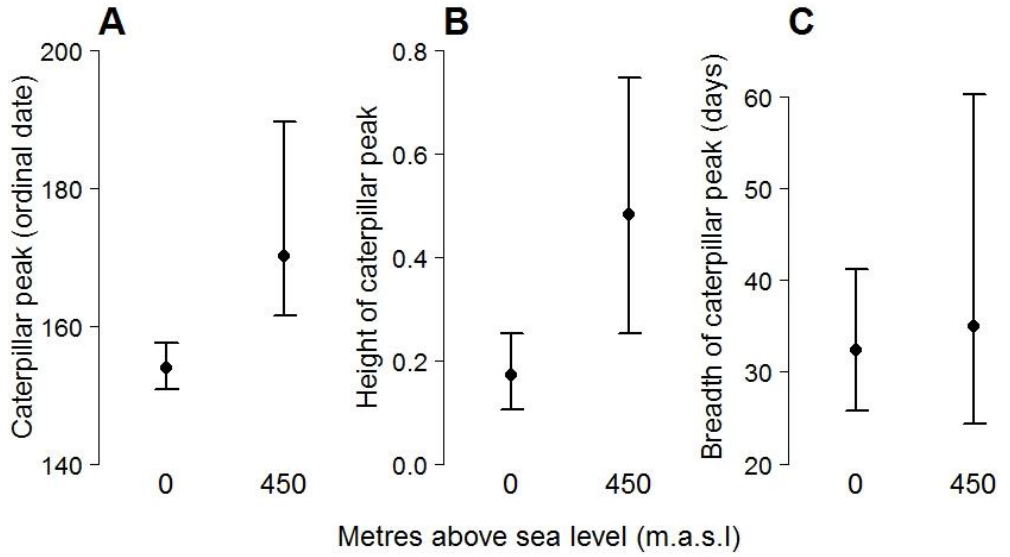

Figure 5.5 The 95% credible intervals for **A** date of caterpillar peak **B** height of the caterpillar peak **C** breadth of caterpillar peak at the extreme elevations of the transect. Predictions are based on the posterior distribution of the GLMM reported in Table 5.3 and depicted in Figure 5.4, in 2014, with all other variables at their mean.

5.4.5 Host tree species effects on the temporal distribution of the spring caterpillar peak

While there were few significant effects of tree species on the timing and shape of the caterpillar peak (Table 5.4), some interesting trends emerge in the predicted temporal distributions (Figure 5.6). Taking birch as a baseline, oak shows a higher but shorter peak sooner after first bud burst (FBB), sycamore shows a lower, shorter peak sooner after FBB and willow shows a higher, longer peak longer after FBB (Figures 5.6 and 5.7). The caterpillar peak on willow was significantly more delayed after FBB than for oak, and oak and willow had significantly higher caterpillar peaks than sycamore. When these timings are converted from days after FBB to ordinal date (referenced to observed FBB at a single site, see 5.3.4), the difference in timing is reduced such that the caterpillar peaks on all trees are more synchronous (Figure 5.7B).

Table 5.4 Tree species and timing predictors of the probability of finding a caterpillar via branch beating, together with 95% credible intervals (CI), estimated from a GLMM (see 5.3.4).

	Slope	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	-1.78	-2.23	-1.38	
Days since FBB	0.069	0.052	0.086	< 0.001 ***
Days since FBB^2	-0.0020	-0.0029	-0.0012	< 0.001 ***
Oak	1.19	0.66	1.72	< 0.001 ***
Sycamore	-0.45	-1.13	0.21	0.18
Willow	-0.013	-0.676	0.611	0.97
Year 2015	-2.02	-2.39	-1.65	< 0.001 ***
Year 2016	-1.00	-1.33	-0.68	< 0.001 ***
Days since FBB : Oak	-0.035	-0.065	-0.005	0.02 *
Days since FBB : Sycamore	0.025	-0.037	0.092	0.46
Days since FBB : Willow	0.020	-0.020	0.057	0.32
Days since FBB^2 : Oak	-0.0011	-0.0027	0.0004	0.17
Days since FBB^2 : Sycamore	-0.0016	-0.0044	0.0009	0.20
Days since FBB^2 : Willow	0.00081	-0.00051	0.00211	0.23
Random Effects				
Site	0.59	0.23	1.02	
Tree ID	0.20	< 0.001	0.43	

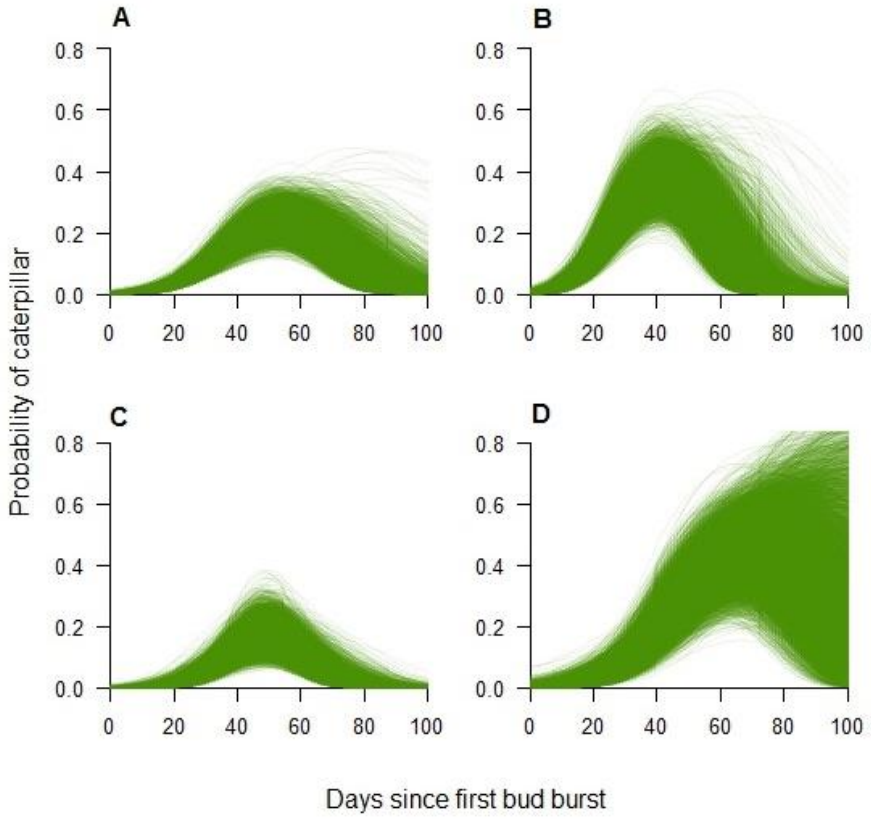

Figure 5.6 Probability of caterpillar occurrence on days after first bud burst for **A** Birch **B** Oak **C** Sycamore **D** Willow. Posterior distributions from GLMM reported in Table 5.4 depicted, in 2014 and with all other variables at their mean.

When I analysed biomass rather than probability of occurrence, the temporal distribution is not humped, but continues exponentially to the end of the study period (Table 5.5). Multiplying the posterior distributions of both models together and accounting for both occurrence and size, I find that the actual peak in caterpillar biomass available in the spring is later (c.10 days) for all analysed tree species than when only caterpillar occurrence is considered (Figure 5.7A). As this differs slightly between tree species, it also gives rise to a more coincident peak between birch, sycamore and oak (Figure 5.7B). The heights and breadths of the caterpillar biomass peak do not differ between species (Figure 5.7C-D). Willow is estimated too poorly by the biomass model to be analysable due to its late occurrence peak resulting in a wide variance in predicted peak dates, which extend long after the end of the study period.

Table 5.5 Tree species and timing predictors of caterpillar biomass obtained via branch beating, together with 95% credible intervals (CI), estimated from a GLMM (see 5.3.4).

	Slope	Lower 95% CI	Upper 95% CI	pMCMC
Fixed Effects				
Intercept	1.76	1.39	2.11	
Time since FBB	0.038	0.014	0.062	0.001 **
Time since FBB^2	0.00021	-0.00087	0.00134	0.70
Oak	1.14	0.58	1.63	< 0.001 ***
Sycamore	0.11	-0.59	0.74	0.75
Willow	-0.20	-0.86	0.49	0.57
Year 2015	-0.95	-1.33	-0.53	< 0.001 ***
Year 2016	0.47	0.12	0.81	0.008 **
Time since FBB : Oak	0.015	-0.020	0.051	0.41
Time since FBB : Sycamore	0.021	-0.037	0.083	0.50
Time since FBB : Willow	-0.050	-0.094	-0.005	0.03 *
Time since FBB^2 : Oak	-0.00051	-0.00247	0.00157	0.61
Time since FBB^2 : Sycamore	-0.0010	-0.0036	0.0013	0.42
Time since FBB^2 : Willow	0.0022	0.0004	0.0040	0.018 *
Random Effects				
Site	0.18	0.03	0.37	
Tree ID	0.038	< 0.001	0.137	

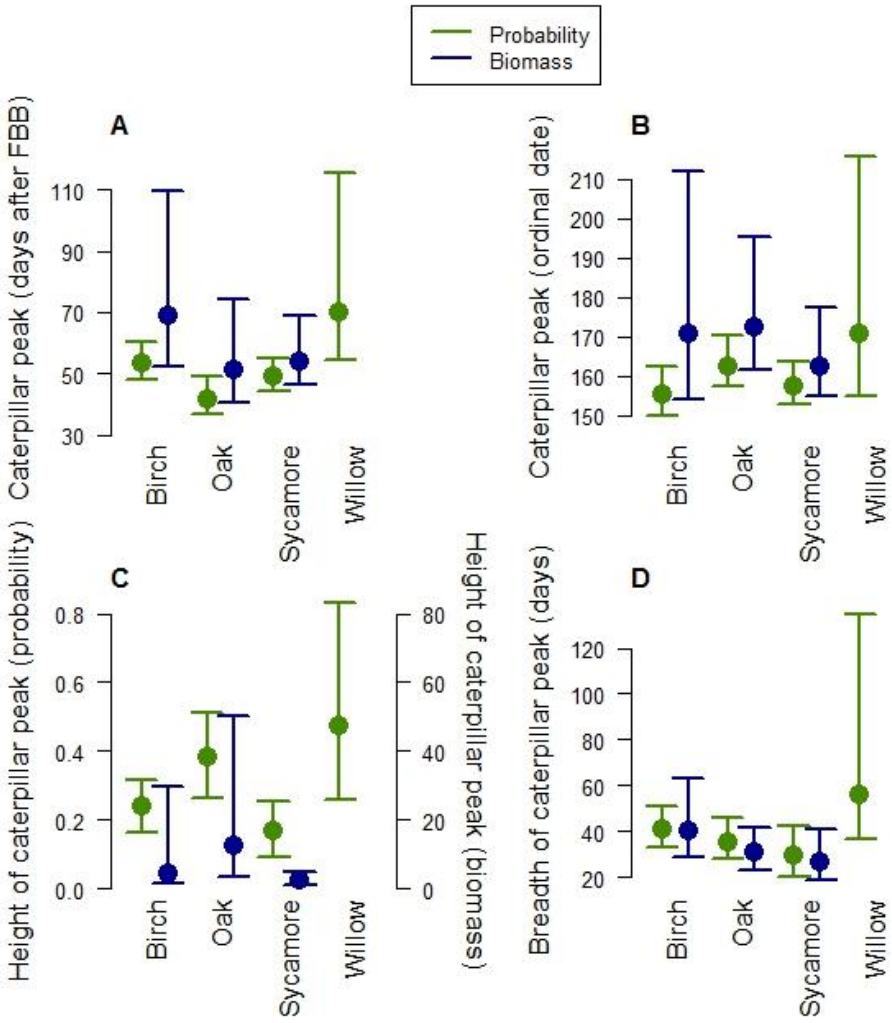

Figure 5.7 The 95% credible intervals for **A** Timing of peak (days after FBB) **B** Timing of peak (ordinal date) **C** Height of caterpillar peak (probability of caterpillar occurrence for probability, biomass peak for biomass) **D** Breadth of caterpillar peak (days) across four tree species. Taken from multiplicative posterior distributions from the GLMMs reported in Tables 5.4 and 5.5, depicted, in 2014 and with all other variables at their mean.

5.5 Discussion

In accordance with other studies (Hunter 1992; Butler & Strazanac 2000; Wesolowski & Rowinski 2006a), the spring caterpillar peak, although diverse in caterpillar species present, was dominated by a select few species, with winter moth accounting for 33% of all caterpillars identified, and over half of all caterpillars collected consisting of just the three commonest species. This provides evidence in support of these few very common species within a landscape being particularly important for their passerine predators at this time of

year (Visser *et al.* 1998; Charmantier *et al.* 2008). Host tree species has a large effect on the availability of caterpillars in spring, including generalist winter moths, with oak and willow having significantly higher chances of having a caterpillar sampled from them than other tree species in Scotland. Whilst biogeography had rather little effect on the likelihood of caterpillar or winter moth occurrence, it had large effects on the temporal distribution of caterpillars, with the peak date delayed by 3.7 days per 100m increase in elevation. In addition, once the identity of tree species was excluded from the model, the height of the peak increased with elevation. This effect can probably be attributed to an increase in the amount of willow at the higher elevation sites (Figure 2.2). Inter-annual variation in caterpillar occurrence was large, but this was less the case for winter moths. There was no effect detected of local tree availability influencing the likelihood of sampling a caterpillar, whereas winter moths were seemingly more likely to be found on a host tree species when that hosts local availability was lower. This study therefore provides evidence that the temporal distribution of caterpillars is geographically variable due to environmental heterogeneity and this should be factored into future trophic mismatch studies operating within this system.

Caterpillar diversity and abundance is known to vary dependent upon host tree species, being higher on native and more abundant tree species in the landscape (Southwood 1961; Fuentes-Montemayor *et al.* 2012). This diversity in host tree quality for caterpillars was corroborated by this study, but I address the question across a much larger range of native and widespread broadleaf trees than previously explored and extend it to include the variance in the temporal distribution of caterpillars in relation to the spring peak for the most common tree species in the study. Oak and willow have previously been identified as hosting the highest caterpillar diversities in the UK (Kennedy & Southwood 1984; Waring & Townsend 2017) and this study extends this to show that they also have the highest likelihood of having a caterpillar sampled from them, which can be interpreted as them hosting the highest caterpillar densities, which was reflected in their higher spring peaks. However, this peak occurred later after budburst in willow and lasted longer, which may be the result of a longer period of leaf palatability, as palatability decreases due to reduced nutritional content of leaves and a build-up in defensive chemicals (Feeny 1970; van Asch & Visser 2007; Forkner *et al.* 2008).

Winter moths were detected on almost all tree species sampled, with oak and willow hosting elevated abundances, supporting previous research indicating that while this species is a generalist feeder, they do show some species preference (Wesolowski & Rowinski 2006a).

Winter moths also seemed to outbreak more on willow at higher elevations (Figure D3 and pers. obs.) along the transect, agreeing with previous research that suggests that outbreaks are more likely to occur at higher elevations (Raymond *et al.* 2002), possibly due to fewer natural enemies. This tendency for higher elevation outbreaks made a substantial difference to increasing the peak at higher elevations, which was non-significant when tree species was included in the model, but was substantial without, as well as increasing the likelihood of finding a caterpillar on willow. Winter moths were also significantly more likely to occur on less abundant host plants within the local environment, which was an unexpected result at odds with the general consensus that caterpillars are more frequent on locally common species (Kelly & Southwood 1999; Wesolowski & Rowinski 2006a) and could also be due to this outbreak tendency on willow at high elevations, where it is not the commonest local tree species. Oak is also often not the commonest local tree species and therefore it may have been more appropriate to test for the interaction between tree species and local availability of that tree species. The broadly temporally coincident caterpillar peaks across tree species with respect to ordinal date, rather than time since budburst of a given tree, could also give credence to the idea that winter moth caterpillar emergence is locally adapted to the most important host tree in a given landscape and they feed on all others opportunistically at this time (Wesolowski & Rowinski 2006a). Another possibility is that they are adapted to an average phenology of various host trees. This finding is also in accordance with Veen *et al.* 2010 who found broadly temporally coincident peaks across deciduous tree species, however birch in the Veen *et al.* 2010 study had an earlier peak, for which I find no evidence, but I only have a weak projection of this by comparison at a single site (STY, Figure 5.7B).

Biogeography was found to have little effect on the probability of finding a caterpillar, with neither latitude nor elevation significantly altering this likelihood, contrary to previous studies that found decreases in caterpillar abundance with increasing elevation and latitude (Garibaldi *et al.* 2011; Smith *et al.* 2011; Pellissier *et al.* 2012). This may be due to the scale of variation assessed in this study being too limited to detect a trend. Elevation did, however, significantly affect the temporal distribution of caterpillars, with the peak date delayed by 3.7 days/100m rise in elevation and increasing caterpillar availability if tree species was disregarded, as this was due to willow being more common at higher elevations. The breadth of the peak was unaffected by elevation. Previous studies have shown a delay in invertebrate (Fielding *et al.* 1999), including caterpillar (Smith *et al.* 2011), emergence in response to increasing elevation, but this is the first study as far as the author is aware to estimate the change in date of the spring caterpillar peak over an elevational gradient. The delay in

caterpillar peak date with increasing elevation is probably due to lower temperatures delaying hatching and growth (Buse & Good 1996; Buse *et al.* 1999; Milonas & Savopoulou-Soultani 2000). Knowledge of how the caterpillar peak changes geographically will be useful when analysing the effects of trophic mismatch on caterpillar predators on a larger geographic scale.

The caterpillar peak date was delayed by c.10 days when the size (volume) of caterpillar was factored in addition to likelihood of occurrence. This was due to caterpillar volume increasing seasonally throughout the study period but probability of occurrence following a hump shaped distribution, which has been shown in other studies (Naef-Daenzer & Keller 1999). From the literature it is clear that both the temporal distribution of caterpillar occurrence and the size of the caterpillars available are important for passerine predators (Naef-Daenzer & Keller 1999; Naef-Daenzer, Naef-Daenzer & Nager 2000) and the product of these two measures is probably the 'true' peak of total caterpillar biomass that is most relevant to insectivorous woodland passerine breeding success (Visser *et al.* 1998; Charmantier *et al.* 2008). Assuming that the rate of frass production corresponds to caterpillar biomass then this peak should correspond to the peak that can be estimated from frass.

In the context of trophic mismatch for insectivorous passerine birds dependent upon this spring caterpillar peak , oak and willow have the highest peaks (Table 5.4, Figure 5.5) and thus could be the most influential for increasing breeding success by hosting the largest available food supply (as depicted by tree A in Figure 5.1B). Willow also appears to have a longer breadth of peak than the other species, which could mean that mismatch in willow-dominated habitats could have less severe consequences than mismatch in other habitats, such as oak. Elevation also affected caterpillar peak date (Figure 5.4), supporting geographical variation in peak date (Both *et al.* 2004b; Smith *et al.* 2011) which could buffer mismatch at a landscape scale, as depicted by the variation between locations A/B:C in Figure 5.1D. Winter moths appear to be the primary constituent of the caterpillar peak across most habitats and locations, supporting previous research emphasising their relative importance for mismatch in temperate European woodlands (Visser *et al.* 1998; Wesolowski & Rowinski 2006a).

It must be recognised however that the specific tree species findings in this study only directly relate to the spring caterpillar peak in Scotland. I did not, for example, detect the

presence of green oak tortrix, a species that has been found to be twice as common on oak as winter moth at Wytham Woods in southern England (Hunter 1990, 1992). If the degree of interspecific competition among caterpillars on a single tree is weak, the presence of green oak tortix may lead to oak being an even richer source of caterpillar abundance in England. In addition, aspen (*Populus tremula*) did not register a single caterpillar (Fig D6) despite being previously noted as having high diversity and palatability (Kennedy & Southwood 1984; Schwartzberg *et al.* 2014). I think that this is primarily due to timing, as aspen is very late in developing its first spring leaves (at the very end of the sampling period) and has new growth throughout the summer, supporting higher caterpillar diversities in late summer than early spring (Niemela & Haukioja 1982; Niemela *et al.* 1982). It could also be due to low sampling effort on this tree species compared with others. However, it is clear that caterpillar diversity and abundance varies with respect to geographic location, time and host tree species, and that all three of these factors interact to form the local spring caterpillar peak.

Utilising a branch beating method for caterpillar sampling allowed me to directly sample, measure and identify the caterpillars on the foliage, providing advantages over other methodologies such as frass fall and half fall, whose relative caveats are discussed in the introduction. However, branch beating does itself also present disadvantages, foremost of which is that by removing sampled caterpillars and resampling branches I may be altering the potential future sampling and peak biomass. The aim of only resampling every four days was to allow time for caterpillar recolonisation in an attempt to alleviate this issue, but whether this is effective remains unknown and requires further testing

In summary, tree species vary in their likelihood of hosting a caterpillar, with even the generalist and almost ubiquitous winter moth occurring more often on certain preferred tree species. However, there appears to be limited variation in caterpillar phenology across tree species, with a similarly timed caterpillar peak across tree species. Increasing elevation significantly delayed the caterpillar peak date yet biogeography had no significant effect on the likelihood of caterpillar occurrence. Conducting this study along a 220km transect incorporating 40 variable field sites allowed me to investigate and quantify these questions to a much finer degree over a much wider geographical area than previously. This study shows that the spring caterpillar peak in temperate deciduous woodlands varies both by host tree species and biogeographically and this should be factored into future studies involving trophic mismatch in this system and the possible demographic effects on caterpillar predators.

Chapter 6

General discussion

Spinningdale

6.1 Overview

Anthropogenic climate change is increasing spring-time temperatures in the northern hemisphere and this is impacting ecology (Walther *et al.* 2002; Parmesan 2006). A key ecological impact has been on the timing of phenological events, which are a sensitive ecological indicator of climatic change (Edwards & Richardson 2004; Thackeray *et al.* 2016). This is consequently altering ecological interactions between trophic levels within communities and may be causing trophic mismatch (Durant *et al.* 2007; Miller-Rushing *et al.* 2010). The deciduous tree – folivorous caterpillar – insectivorous passerine spring-time food web has been important in uncovering the effects of climate-mediated phenological change and trophic mismatch (Visser *et al.* 1998; Both *et al.* 2006).

The principal aim of my thesis was to enhance our understanding of how phenology, community dynamics and their consequences may vary geographically in this woodland system. I aimed to expand the current single-species food chain studied at single sites with similar habitats into a more realistic multi-species, geographically variable food web in order to form more accurate predictions about how phenological change and trophic mismatch will affect organisms in this system at a landscape scale under future climate change scenarios. In order to study this, I established a novel 220km, 40 site transect across Scotland and explored how biogeographic and habitat variation operates in this system, from the community composition and temporal distribution of caterpillars, through adult blue tit diet, to blue tit occupancy and productivity. I also attempted to disentangle the environmental predictors of blue tit reproductive phenology as this knowledge is crucial to being able to predict how blue tit phenology will react to a changing climate and impact any subsequent trophic mismatch. Developing faecal metabarcoding methodology for adult blue tit faeces also enabled me to identify spring-time blue tit diet to a hitherto unattainable level of taxonomic resolution.

6.2 Environmental predictors of blue tit reproductive phenology

Establishing the environmental predictors of woodland passerine reproductive phenology is an essential first step in being able to predict future phenological changes (Visser *et al.* 2002). Studies have found that temperature (Schaper *et al.* 2012; Phillimore *et al.* 2016) and tree phenology (Nilsson & Källander 2006; Bourgault *et al.* 2010) both correlate with egg

laying, and that artificially increasing food abundance (Svensson & Nilsson 1995; Robb *et al.* 2008a) and photoperiod (Silverin *et al.* 1989; Lambrechts & Perret 2000) can advance laying, but all have analysed predictors singly and lack a unified view. Also, as many of these potential predictors vary in similar patterns at single sites in the wild, a correlation between one predictor and phenology may not indicate that this predictor variable directly affects reproductive phenology, as it could actually dependent on another, mediator variable. In simultaneously modelling how temperature, tree phenology, prey abundance and photoperiod affect two measures of blue tit reproductive phenology (the onset of nest building and first egg date), I attempt to unravel the relative importance of each and provide the first single-model estimate analysing all potential environmental predictors of woodland passerine reproductive phenology. By considering the separate effects of night time and day time temperatures, I also uncovered which temperatures the birds were most responsive to a finer detail than previously known.

All potential predictors significantly predicted blue tit reproductive phenology when treated independently, however in the multi-predictor models night-time temperature was the most important significant predictor of both phenological responses, and the only significant predictor of nesting onset. This lends weight to the argument that temperature causally affects reproductive phenology rather than acting through an intermediary factor (Visser *et al.* 2009). That night-time temperature significantly outperformed day-time temperature as a predictor implies that minimum temperatures are affecting phenology rather than maximum; supporting a thermal constraint to egg laying (Stevenson & Bryant 2000). If this novel insight is upheld in subsequent analyses on other datasets (e.g. British Trust for Ornithology nest record data), it will reform and refine our understanding of how blue tits time their breeding, and greatly improve the accuracy with which future phenological predictions in this system can be made. Tree phenology did not significantly predict blue tit reproductive phenology in the multi-predictor models and this suggests that previous studies finding it to be a significant predictor (e.g. Nilsson & Källander 2006) may be spurious and that the relationship identified is due to tree phenologies co-correlation with temperature.

Besides night-time temperatures, the other significant predictor of lay date was invertebrate prey abundance, and the faecal metabarcoding (Chapter 4) confirms that the invertebrates collected on the sticky traps are indeed prey items. Theoretically, this predictor could combine with temperature to form a coherent signal of energetic constraint on egg laying imposed by both energy income (prey availability) and expenditure (cold temperatures). It

also highlights a mechanism that may facilitate the fine-tuning of timing after the onset of nesting, as fine-tuning in other stages of reproductive phenology before (Cresswell & McCleery 2003; Simmonds *et al.* 2017). I also considered a potential predictor that has been hitherto unstudied – that of a specific dietary cue – and identified the increasing importance of *Lepidoptera*, *Hemiptera* and *Coleoptera* in the diet as egg laying nears. It is especially interesting to note the presence in the diet at this stage of spring of winter moth, northern winter moth and scarce umber, presumably as early instar caterpillars. These species were the most abundant caterpillars encountered along the transect later in spring, forming the bulk of the caterpillar peak that is so important for nestling fitness, with winter moths known to be particularly important (Visser *et al.* 1998; Wilkin *et al.* 2009). It was thought that they were not present in the environment at the time when passerines needed to commence reproductive phenology, and therefore they required an environmental predictor to act as a proxy to allow them to time their reproduction to maximise fitness (Caro *et al.* 2013). However, it is conceivable that the presence of these species in the diet at this time signifies the possibility of a direct cue to initiate egg laying, with the early caterpillar instars providing a reliable indicator of when caterpillar peak biomass will occur. This would allow for very accurate tracking of spring caterpillar peaks by the birds and alleviate much worry about future trophic mismatch.

Whilst these insights into the environmental predictors of blue tit reproductive phenology represent a significant advancement in our knowledge, they do not yet provide the exact mechanistic understanding we require to make accurate future predictions. Further research is required into assessing the possibility of a direct cue provided by early instar caterpillars. This could be achieved by an environmental survey attempting to find these prey taxa in the environment and further dietary studies quantifying the diet year-round to analyse their importance at the stage of egg laying. Alternatively, they could be ever-present in the diet in various forms (e.g. caterpillar, pupa, adult) all year round. In addition, research on other datasets is required to ascertain the ubiquity and accuracy of night-time temperatures as an environmental predictor of reproductive phenology. A further limitation is that, although we assume blue tits to be a model insectivorous woodland passerine, it is unknown if they utilise the same environmental predictors as others, such as great tits and pied flycatchers, which are also believed to be reliant on the same prey peaks (Charmantier *et al.* 2008; Burger *et al.* 2012). Assessing the uniformity of the predictors we highlight across these species is necessary to provide accurate future predictions at the ecosystem level.

6.3 Implications for trophic mismatch

Climate-induced trophic mismatch reduces insectivorous passerine fitness through increasing phenological asynchrony with the critical spring caterpillar peak (Visser *et al.* 1998, 2006). However, the overwhelming majority of studies have been conducted at single-site mature oak-dominated climax woodlands and the findings generalised across entire populations and woodland habitats (Both *et al.* 2004a). My thesis provides a primary quantification of geographic and habitat variation in this system and attempts to establish a baseline of how this variation operates to inform future landscape scale predictions of trophic mismatch theory in this system and ensure that implications are extrapolated in a more reliable and accurate way. This study provides the most comprehensive insights into the composition of the caterpillar peak to date, identifying the species that contribute and analysing how the peak varies geographically and by habitat. I also analyse how nest site occupancy and productivity vary over the same axes, providing a reference point and yardstick into underlying variation before mismatch is accounted for (Visser & Both 2005). I also quantify how trophic interactions (diets) vary and consider how this may affect community dynamics.

Geographical variation in the focal system is pronounced, with nest site occupancy showing dramatic elevational and latitudinal clines, diet turning over across sites and elevation significantly retarding the date of the spring caterpillar peak. Elevation and latitudinal trends in dietary β-diversity are large, as is site-to-site variability. This variation in diet could represent differing local prey communities (Kennedy & Southwood 1984), and if this also occurs during the breeding season, could drastically alter the trophic interactions involved in trophic mismatch, and alter the potential buffering afforded by dietary switching to other prey sources (Burger *et al.* 2012). As dietary turnover is greater over the elevational gradient used in this study than the latitudinal gradient, it could also be that the species interactions involved in generating trophic mismatch could vary more over this axis too. Similarly, habitat variation in the system was large, with tree species affecting both caterpillar abundance, as found in some other studies (Wesolowski & Rowinski 2006a; Veen *et al.* 2010) but on a finer, intra-deciduous scale, as well as fledging success. Contrary to what one might expect, the effects of tree species on caterpillar diversity and fledging success were not identical, as although oak provided excellent habitat for both caterpillars and birds, willow hosted many caterpillars but was the only individually analysed tree species not found to promote productivity. This could be due to other factors, such as the positive correlation between elevation and willow abundance, or a possible methodological bias towards willow

in caterpillar collection, as noted in 5.5, providing an overestimate of the caterpillar abundance in willow. However, the fact that there is marked geographic and habitat variation, that there is an incongruence between blue tit reproductive decisions (e.g. occupancy, clutch size) and outcomes (i.e. productivity), and that there is a difference between habitats that promote caterpillar productivity and those that promote bird productivity, all highlight that insights gained at one site cannot necessarily be assumed to extrapolate to others across a species' range.

My thesis also emphasises the overwhelming dominance of a small handful of caterpillar species contributing toward the spring caterpillar peak (Hunter 1990; Wesolowski & Rowinski 2006a). I also show that the identity of dominant species varies geographically, as I found no evidence of the commonest species in an English oak wood, green oak tortrix (Hunter 1990, 1992), and instead identified the importance of scarce umber in birch habitats. These common caterpillar species, and in particular the seemingly ubiquitous winter moth (Wesolowski & Rowinski 2006a; Waring & Townsend 2017), should be considered 'keystone' species in this ecosystem and if something happens to these species the knock-on effects could be large throughout the ecosystem. Assessing their relative importance and geographic variability in the diet of nestling blue tits could help quantify their significance.

6.4 Molecular scatology

Molecular scatology, such as the faecal metabarcoding employed in this thesis, has the potential to illuminate many ecological questions through the reliable identification of even cryptic diets (Pompanon *et al.* 2012; Taberlet *et al.* 2012). Despite this technique offering ample promise, it has rarely been used for avian studies or on large sample sizes of any organism, and never in this system. I show in this thesis how next generation techniques such as this can shed new light on even well-studied organisms (Betts 1955), and the new tools developed here, from novel collection techniques through improved lab techniques to new prey diversity analyses, will hopefully enable and encourage further use of these techniques, particularly on adult birds. I demonstrate that the method is robust even when dealing with fairly old samples of unknown origin and that repeatability of taxa identified as present from separate DNA extractions of the same sample is fairly high, contrary to the only previous estimate of this (Jedlicka *et al.* 2017).

Whilst there are plentiful benefits afforded by faecal metabarcoding, this thesis also identifies some potential caveats. Control samples have rarely been employed in previous studies (but see De Barba *et al.* 2014), yet were invaluable in allowing me to identify quality cut-off levels and contamination and should be included as standard in future research. Contamination is an issue to consider, with both systematic contamination and remnant background levels identified. I have learned that randomisation of samples on PCR plates is therefore necessary to avoid the worst effects of this, making it easier to estimate plate effects. Quality control of resultant prey taxa is also essential as large numbers of output MOTUs based on the standard 2% similarity threshold correspond to the same taxon and not acknowledging this would substantially artificially inflate dietary diversity, alongside high numbers of misread 'noise' MOTUs which do not relate to genuine prey items. The method also relies on well-defined potential environmental prey DNA reference databases, which may not currently be applicable for highly diverse biomes or understudied regions (Coghlan *et al.* 2013; Sedlock *et al.* 2014). Faecal metabarcoding also works substantially better on animal prey than either plant or fungi, due to the standardised and diverse CO1 mitochondrial barcoding gene allowing fine and consistent taxonomic resolution (Clare 2014b; Kress *et al.* 2015). Probably the primary drawback with this technique, however, is the lack of reliable quantification of content per sample precluding analysis of how common prey species are per sample (Deagle & Tollit 2007). This has not been tested thoroughly and should provide a fruitful area for future research, as if this could be quantified it would substantially increase the value and application of this technique.

6.5 Transects as a method for phenological study

In order to address the aims in this thesis, I set up a 220km transect incorporating 40 field sites across Scotland. As far as I am aware, this is a unique approach to studying phenology in this system and allowed me to generate standardised data across a wider geographic area than previous studies. The transect methodology was also essential to integrating sufficient levels of geographic and habitat diversity in the study, with a far wider diversity than could be achieved at a single site or over a small range of sites and enabling me to answer questions on geographic and habitat variability in the system. The habitats included along the transect are highly consistent with those found in Scotland and the UK as a whole (Forestry Commission 2013) and facilitate a more accurate representation of situations encountered by the average blue tit in the UK than solely studying climax oak woodlands (Charmantier *et al.* 2008), which are rare across Scotland. This should provide a more accurate interpretation as

to what is occurring in this system at the landscape- and population- levels. Additionally, collecting data over a wide geographic area opens up the possibility for space-for-time substitution (Phillimore *et al.* 2012), as discussed in 1.4. A final benefit of the transect methodology is that it allowed me to disentangle environmental predictor variables that vary in a similar fashion at individual sites, but are less autocorrelated when compared across multiple sites.

Altogether, the transect methodology was highly successful and accessed novel research avenues in this system; however, there are some limitations. Firstly, it is logistically and financially far harder to collect data from a transect of field sites rather than a single site. This also imposes greater time constraints to data collection due to travel time, limiting the data collectable at individual sites and therefore providing only poor estimates of certain variables at each site when compared to data collection possible at single sites. This was most noticeable when estimating the caterpillar peak at each site within each year, which due to logistical restraints, was imprecisely estimated, making it impossible to study mismatch directly with only three years of data. This issue will be overcome in future with more years data allowing better predictions of peak caterpillar dates. A further issue inherent in all studies incorporating space-for-time substitution is that the patterns observed over space may not be equal to those governing variation over time and therefore possibly violating a key assumption (see 1.4). Lastly, while 40 independent data points per year (sites) for many variables provided me with sufficient statistical power to assess trends over a wide geographic area, fewer would almost certainly have been insufficient and this should be considered when establishing any future transect study of mismatch, as the site is the level that most studies should be statistically analysed at to avoid pseudoreplication.

6.6 Future research directions

In addition to those outstanding questions mentioned above, this thesis highlights further potential future research directions. Firstly, I individually counted nearly one hundred thousand dead invertebrates on the sticky traps used in this thesis and identified each to a minimum of order level resolution. These data could be properly analysed to identify major geographic and habitat patterns in the abundance and phenology of various invertebrate groups, including *Auchenorrhyncha*, *Heteroptera* and *Sternorrhyncha* (all *Hemipteran* suborders), *Coleoptera*, *Hymenoptera* and *Nematocera* and *Brachycera* (*Dipteran* suborders). To the best of my knowledge this has not been conducted before (but see

Southwood *et al.* 2004), and will complement the work in chapter 5 on *Lepidopteran* caterpillar geographic and habitat variability to provide a greater interpretation of blue tit temporal prey availability and woodland community composition, which could then be linked to the diet discovered in chapter 4, as all of these groups were found to feature in the diet. Secondly, before egg laying commenced, nest heights were measured on every visit throughout the spring by measuring the depth of the nesting material from the outside of the bottom of the nest box (front opening) to the top of the main mass of nesting material, excluding stray strands. These measurements could be related to temperature and other collected variables to identify the conditions that promote nest building behaviour, as another measure of phenology in addition to those analysed in chapter 2. Thirdly, the faecal metabarcoding conducted in chapter 4 also provided data at the 16S and rbcL genetic loci in addition to COI. At 16S I amplified prey items in addition to blue tit DNA and the prey taxa could be compared to CO1 to ascertain overlap in prey taxa and whether the loci are complementary. The rbcL dietary plant data enables analysis of whether blue tits are using the habitat generally or predominantly choosing to forage upon certain tree species in the landscape during the spring as the habitat survey provides us with the contribution of each tree species at each locality. In addition, through preliminary checks, the rbcL data contains widespread evidence of human artificial feeding (e.g. peanuts, sunflowers) at even remote sites along the transect (I imagine some of the remotest occupied blue tit nesting sites in the UK) and a distance decay of supplemental food in the diet to nearest feeder would uncover over what distance human supplementary feeding is impacting blue tit, and subsequently woodland, ecology (Robb *et al.* 2008a). This could have important ramifications for interpreting the multitude of artificial feeding experiments conducted in the UK (Robb *et al.* 2008b; Harrison *et al.* 2010), and the possible effect that artificially elevated blue tit survival could have on competitors.

Nestling faeces were collected from every possible brood at days 6 and 12 over the entirety of the study duration and these could undergo faecal metabarcoding. Analyses could target the prey groups that are most important, and ask whether this is variable and whether these dietary items tie in with the caterpillars that we collect. The similarity to adult diet, whether diet affects weight or fledging success and whether dietary plasticity can counteract trophic mismatch could also be approached. With more years data collection on the transect, trophic mismatch can also be directly assessed and the geographic and habitat variability quantified. This would also benefit from a greater estimation of the caterpillar peak at a site within a given year and this could be achieved by extra branch beating, particularly sampling the

canopy of trees as community composition may be different to that lower down (Forkner *et al.* 2004; Lill *et al.* 2006) and this is where the blue tits primarily forage (Gibb 1954; Perrins 1979).

Away from the transect, a pure aspen stand of suitable size would aid further in distinguishing the environmental predictors of reproductive phenology. Aspen comes into leaf very late in the year, after blue tits normally hatch, and there is also evidence that the caterpillar peak on Aspen is particularly late (Niemela & Haukioja 1982; Parry, Spence & Volney 1998). This would create exceptional tree phenology and prey phenology which is highly divergent from other habitats which would aid in distinguishing their effects from temperature and photoperiod as well as each other. The aspen stands currently on the transect are all intermingled with birch and other tree species, which provide the blue tits alternate foraging opportunities. Unfortunately, as the RSPB will not allow us access to their Insh Marshes reserve, no such suitable pure Aspen stand exists along the transect, or indeed in the UK, but this could be achievable in Scandinavia, or even in North America with a different species of aspen (*Populus tremuloides*) and bird species. Another area of possible future research away from the transect could be faecal metabarcoding from multiple bird species at a large single site study. Many co-existing species are thought to target the self-same caterpillar peak for reproductive success, including other tit species (e.g. great *Parus major* (Visser *et al.* 1998), marsh *Poecile palustris* (Wesolowski 1998)), flycatcher species (e.g. pied *Ficedula hypoleuca* (Both *et al.* 2004a), collared *Ficedula albicollis* (Bauer *et al.* 2010)), woodpecker species (e.g. great spotted *Dendrocopos major* (Smith & Smith 2013), lesser spotted *Dryobates minor* (Wiktander, Olsson & Nilsson 2001)) and warbler species (e.g. wood *Phylloscopus sibilatrix* (Mallord *et al.* 2016), willow *Phylloscopus trochilus* (Hedlund *et al.* 2015)), amongst others. Faecal metabarcoding of nestlings from broods of each at a single site would highlight whether this is indeed true and quantify to what extent they are competing for resources.

6.7 Concluding remarks

This thesis contributes to the knowledge of phenology and community ecology in spring-time temperate deciduous woodlands, as well as developing methodologies such as transect approaches and faecal metabarcoding. The findings of chapter 2 form an essential baseline understanding of how blue tit occurrence and productivity varies geographically and by habitat, from which the effects of trophic mismatch at a landscape- and population- scale can

be more accurately estimated. The analyses in chapter 3 provide new insights into the environmental predictors of blue tit reproductive phenology from which to make more accurate predictions of how phenology will alter under future climate change scenarios. Chapter 4 provides the most detailed description of a wild birds' diet to date, and the faecal metabarcoding and associated methodologies developed in this chapter may help provide answers to many other avian dietary questions in future. Results in chapter 5 identify the keystone caterpillar species contributing to the spring caterpillar peak and demonstrate how the peak date varies geographically. Overall, this thesis advances this focal study system from a single-site, single-species food chain into a more biologically realistic geographically variable food web. It also establishes a transect of field sites across Scotland which can be used as a platform for future questions into, and a better understanding of, climate-mediated trophic mismatch in temperate deciduous woodlands in spring.

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Appendix A

Supplementary material for Chapter 2

Figure A1 Site-level biogeographic patterns in habitat variables.

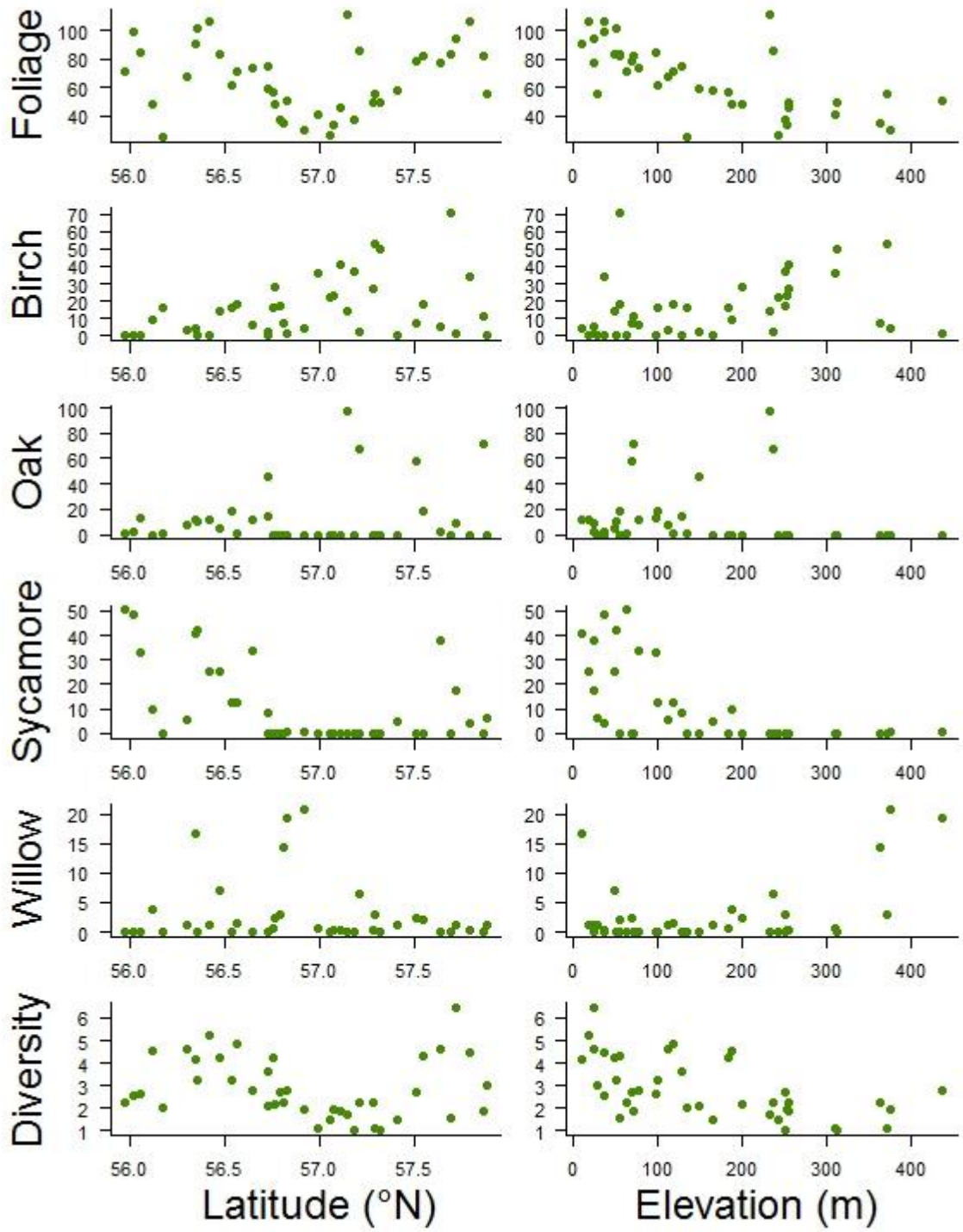

Figure A2 Raw numbers of invertebrates sampled from sticky traps at each site in each year. Some counts exceed the limits of the constant y axis used for comparison purposes.

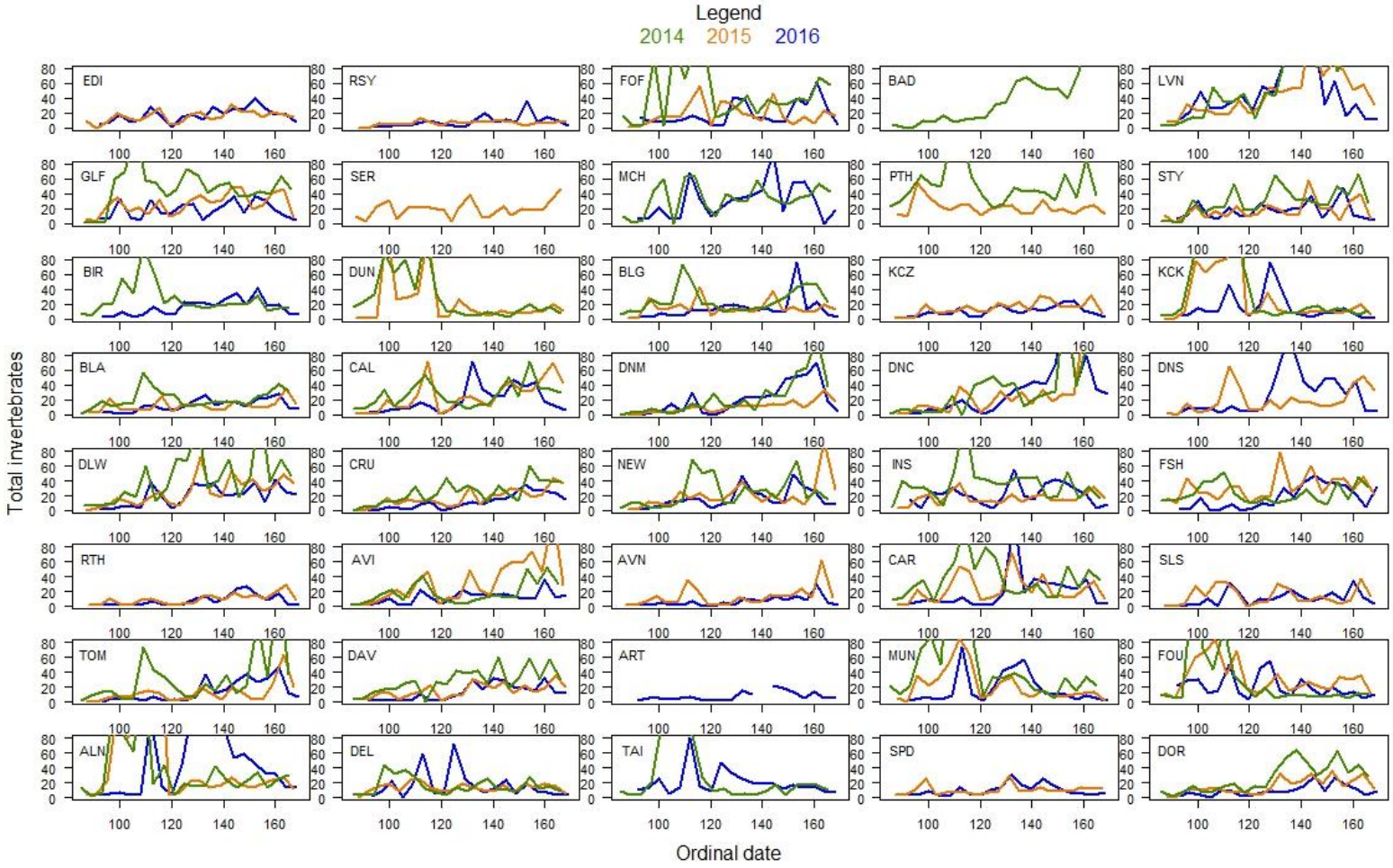

Figure A3 Site level predictions (ln-scale) of total invertebrate numbers from a GLMM (see section 2.3). **A** Early season total invertebrates by latitude **B** Early season total invertebrates by elevation **C** Late season total invertebrates by latitude **D** Late season total invertebrates by elevation.

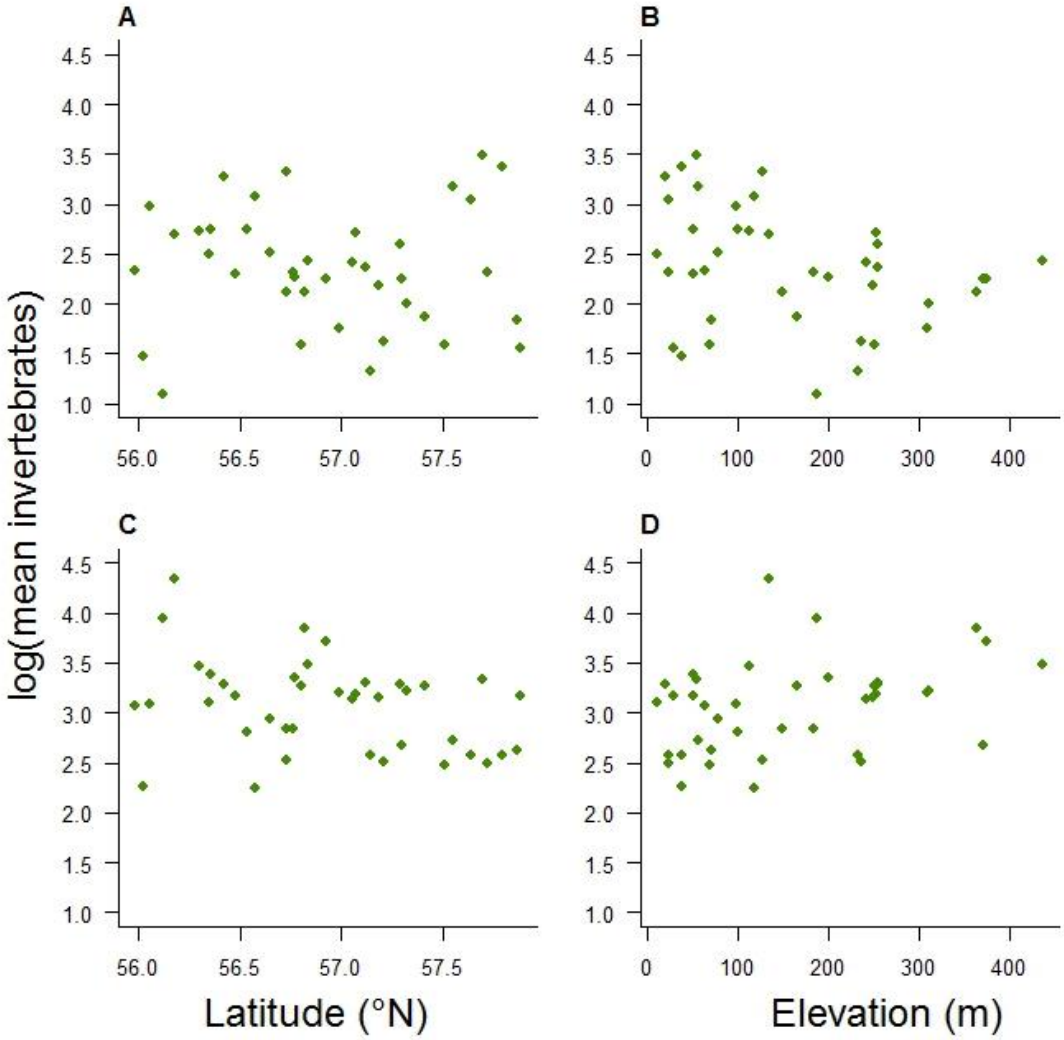

Table A1 A-C Effects on blue tit fledging success along the transect once the analysis is split into the constituent years, to compare with Table 2.3C (showing the result for all years). **D** Effects on total number of fledglings, as opposed to fledging success as a proportion of clutch size (Table 2.3C). Slopes (coefficient) are shown with their associated standard errors (se) from GLMM's.

		A. 2014	**B. 2015**	**C. 2016**	**D. Total Fledglings**
Fixed Term		**coefficient ± se**	**coefficient ± se**	**coefficient ± se**	**coefficient ± se**
Intercept		2.32 ± 0.38	-0.45 ± 0.36	1.20 ± 0.30	1.92 ± 0.05
Total Foliage		-0.0029 ± 0.0229	-0.0097 ± 0.0265	0.025 ± 0.023	-0.00083 ± 0.00283
Birch		0.0029 ± 0.0243	0.033 ± 0.026	0.020 ± 0.027	0.0067 ± 0.0032 *
Oak		0.073 ± 0.056	0.082 ± 0.026	0.029 ± 0.022	0.011 ± 0.003 ***
Sycamore		0.062 ± 0.030	0.053 ± 0.039	0.047 ± 0.035	0.011 ± 0.004 **
Willow		-0.031 ± 0.114	-0.20 ± 0.07	0.10 ± 0.07	-0.00032 ± 0.00794
Tree Diversity		0.33 ± 0.27	0.77 ± 0.35	0.33 ± 0.33	0.10 ± 0.04 **
Latitude		0.57 ± 0.74	0.52 ± 0.35	0.60 ± 0.71	0.038 ± 0.085
Elevation		0.0045 ± 0.0064	0.0084 ± 0.0051	0.011 ± 0.005	0.0015 ± 0.0006 **
Late Invertebrates		1.85 ± 0.96	2.07 ± 0.81	1.92 ± 0.82	0.39 ± 0.10 ***
Blue Tit Density		-3.62 ± 1.65	1.45 ± 1.65	1.53 ± 1.42	0.090 ± 0.159
Year	2015	-	-	-	-0.71 ± 0.07 ***
	2016	-	-	-	-0.30 ± 0.06 ***
Random Term		**variance**	**variance**	**variance**	**variance**
Space		3.0×10^{-9}	0.3	2.2×10^{-8}	6.1×10^{-9}
Nestbox ID		3.3	7.1	7.3	0.07
Spatial Autocorrelation		**parameter**	**parameter**	**parameter**	**parameter**
nu		0.5	0.5	0.5	0.5
rho		5.1	82.8	136.6	4.75

Appendix B

Supplementary material for Chapter 3

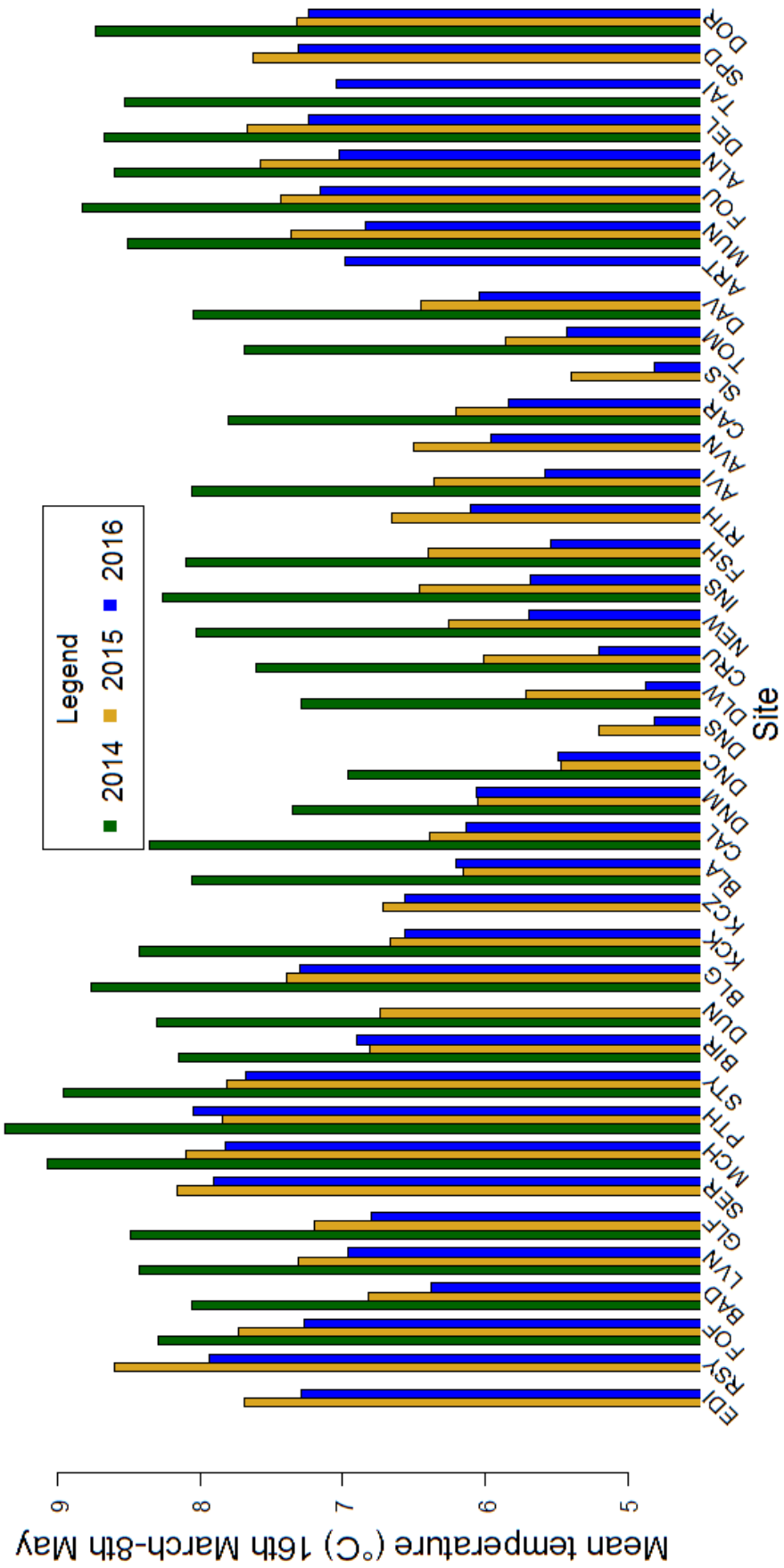

Figure B1 Illustrating mean overall temperatures for the period temp_i across all sites, from south to north when left to right (Table 2.1).

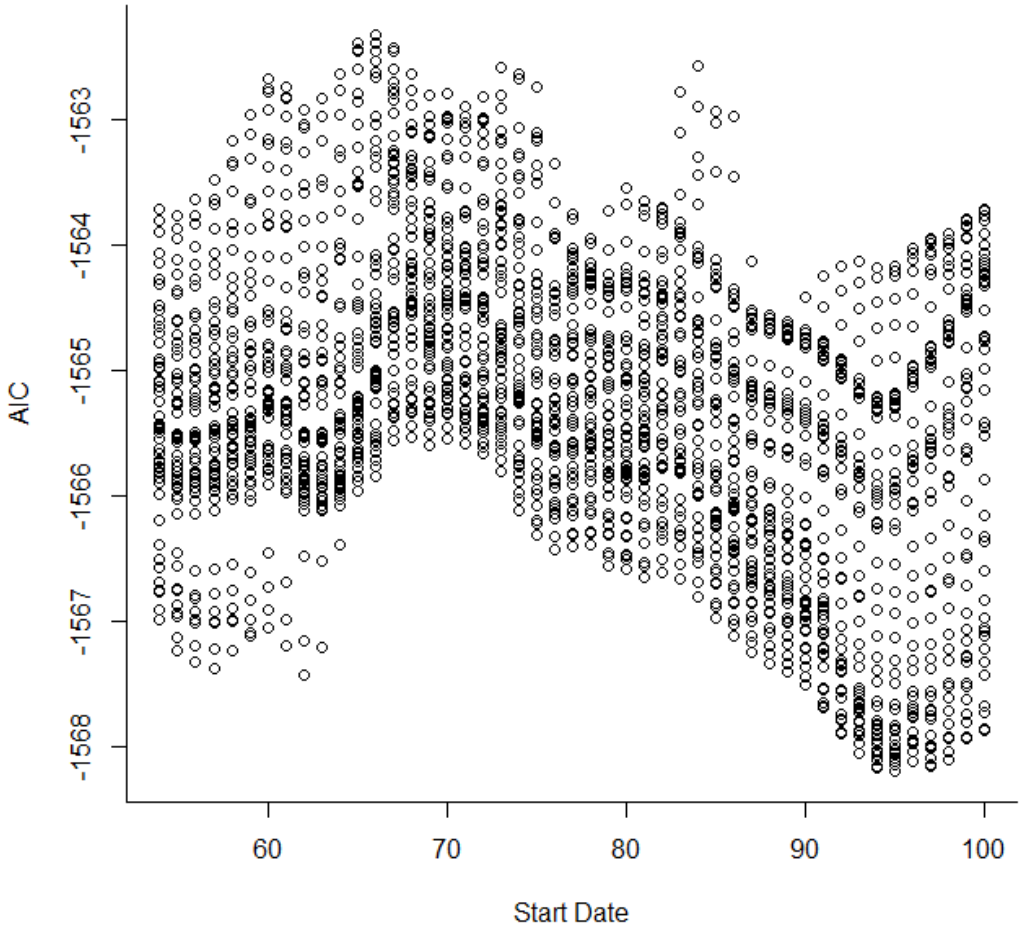

Figure B2 AIC likelihood surface from sliding windows assessing the dates over which temperature best predicts N1.

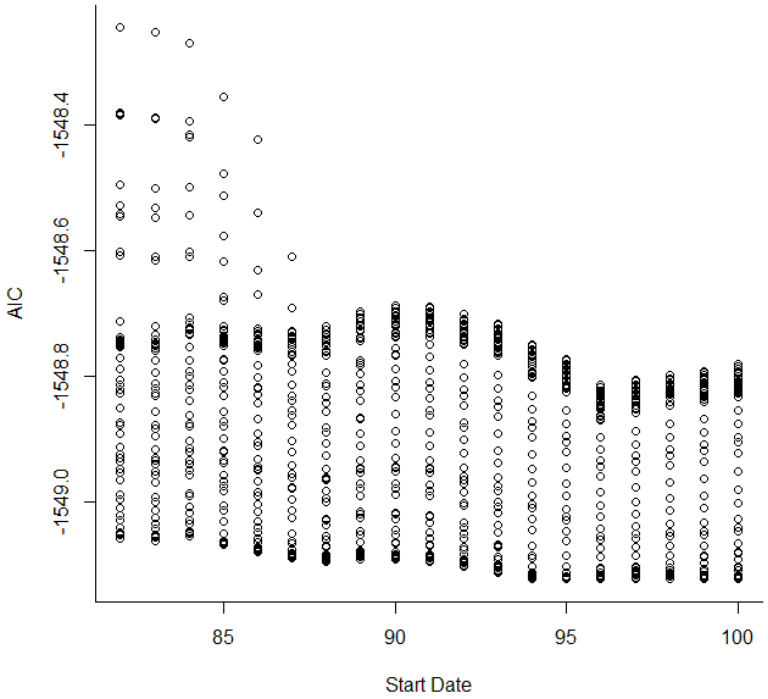

Figure B3 AIC likelihood surface from sliding windows assessing the dates over which invertebrate numbers best predict N1.

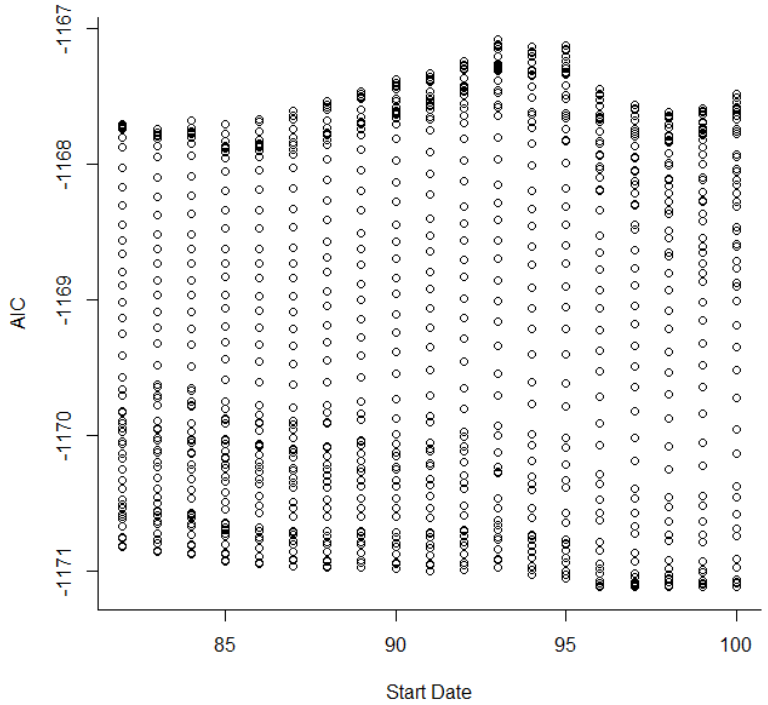

Figure B4 AIC likelihood surface from sliding windows assessing the dates over which invertebrate numbers best predict FED.

Appendix C

Supplementary material for Chapter 4

Section C1 DNA extraction from blue tit faeces stored in ethanol (protocol supplied by Dr James Nicholls).

Methodology uses the Qiagen QIAamp DNA Stool Kit, following the "Isolation of DNA from Stool for Pathogen Detection" protocol (June 2012 edition), with some modifications following Zeale *et al*. 2011 (Mol. Ecol. Res. 11: 236-244) and custom modifications to accommodate dried avian faeces.

1. Remove ~100-200 mg of faeces (typically 2-3 small fragments of faeces, each ~5 mm long) from storage tube, allow ethanol to evaporate off, and then place into a 2 mL round-bottomed centrifuge tube (Eppendorf 2 mL SafeLock tubes are good). Avoid using faeces that has lots of uric acid on it, or scrape off uric acid.

2. Add 1.4 mL Buffer ASL to the faecal sample. Vortexing will not typically homogenise dried avian faeces, so to homogenise add one 3 mm diameter tungsten carbide bead and shake on a Qiagen TissueLyser for 1 minute at 24 Hz.

3. Add 20 μL of Proteinase K (using stock supplied in Stool kit). Vortex briefly to mix, then heat the suspension for 30 minutes at 70 ^{o}C. Both adding ProtK at this step and homogenising using the TissueLyser in the previous step increase DNA yields.

4. Vortex for 30 seconds, then centrifuge sample at 13,000 rpm for 1 minute to pellet faecal particles.

5. Pipet 1.2 mL of the supernatant into a new 2 mL centrifuge tube. The remaining faecal material can be stored and used for microscopic analysis if required; otherwise discard the pellet but don't forget to retrieve tungsten carbide bead first.

6. Add 1 InhibitEX tablet to the sample and vortex immediately and continuously for 1 minute or until the tablet is completely suspended. Incubate suspension for 1 minute at room temperature to allow inhibitors to absorb to the InhibitEX matrix.

7. Centrifuge sample at 13,000 rpm for 3 minutes to pellet inhibitors bound to InhibitEX matrix.

8. Pipet all the supernatant (typically 400-600 μL) into a new 1.5 mL centrifuge tube and discard the pellet. Centrifuge the sample at full speed for 3 minutes. Transfer of small quantities of pellet material will not affect the procedure.

9. Pipet 20 μL of Proteinase K (either from kit, or a user-supplied 10mg/mL solution) into a new 1.5 mL centrifuge tube.

10. Pipet 400 μL of supernatant from step 8 into the 1.5 mL tube containing proteinase K.

11. Add 400 μL of Buffer AL, and mix well by vortexing for 15 seconds. Don't add the proteinase K directly to buffer AL.

12. Incubate at 70 ^{o}C for 15 minutes.

13. Add 400 μL of ethanol (96-100%) to the lysate and mix well by vortexing. Centrifuge briefly to remove any liquid from the lid of the tube.

14. Carefully apply 600 μL of the lysate to a QIAamp spin column (in a 2 mL collection tube) without moistening the rim. Centrifuge at 13,000 rpm for 1 minute. Place spin column in a new 2 mL collection tube and discard the tube containing the filtrate.

15. Repeat step 14 using the remaining liquid from step 13.

16. Carefully open the spin column and add 500 μL of Buffer AW1. Centrifuge at 13,000 rpm for 1 minute. Place spin column in a new 2 mL collection tube and discard the tube containing the flow-through.

17. Carefully open the spin column and add 500 μL of Buffer AW2. Centrifuge at 13,000 rpm for 2 minutes. Discard tube containing the flow-through.

18. Place spin column in a new 2 mL collection tube. Centrifuge at 13,000 rpm for 1 minute. Discard tube containing flow-through.

19. Transfer the spin column into a new 1.5 mL centrifuge tube. Using a low-bind tube will minimise DNA loss through absorption to tube walls (Eppendorf DNA LoBind tubes are good). Pipet 50 μL of Buffer EB (not supplied in kit; EB = 10 mM Tris) directly onto the spin column membrane. Incubate for 5 minutes at room temperature, then centrifuge at 13,000 rpm for 1 minute to elute DNA.

Section C2 Final primer sets used for blue tit faecal metabarcoding (protocol supplied by Dr James Nicholls).

COI:
LepF1, used as is from (Hebert *et al.* 2004)
ZBJ-ArtR2c-deg, a modified version of the primer ZBJ-ArtR2c presented in (Zeale *et al.* 2011). Modifications introduced by me, involving degeneracy at third codon positions towards the 3' end of the primer using data from (Clarke *et al.* 2014) and (Piñol *et al.* 2014).

16S:
16S1F-deg, used as is from (Deagle *et al.* 2007).
Ins16S_1R, used as is from (Clarke *et al.* 2014).

rbcL:
rbcL1 and **rbcLB**, both originally from (Palmieri *et al.* 2009), and assessed for utility as minibarcode primers by (Little 2014).

For metabarcoding, amplicons are produced using a two stage PCR. The initial PCR uses the locus-specific primers with 5' tails containing part of either the Illumina Nextera i5 or i7 adaptor sequence. Reagent concentrations, annealing temperatures and number of PCR cycles vary by locus (see table below). The second PCR uses primers containing the remainder of the respective Nextera adaptor including an 8 base pair index (following the published i5 and i7 indicies used in the Nextera XT kit). This indexing PCR uses the same conditions for all loci, with the exception of the number of cycles (see table below).

LepF1 has the 5' tail TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
ZBJ-ArtR2c-deg has the 5' tail GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

16S1F-deg has the 5' tail TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG

Ins16S_1R has the 5' tail GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

rbcL1 has the 5' tail TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
rbcLB has the 5' tail GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

Indexing primers for the second PCR are:
i7 adaptor primer:
CAAGCAGAAGACGGCATACGAGAT<i7>GTCTCGTGGGCTCGG
i5 adaptor primer:
AATGATACGGCGACCACCGAGATCTACAC<i5>TCGTCGGCAGCGTC

I use Herculase II Fusion polymerase (Agilent catalogue number 600679) for all PCRs, using 0.1μL of polymerase and 1μL of DNA extraction in a 10μL reaction. For the second indexing PCR I use 1μL of template from the first PCR in a 10μL reaction. Final concentrations of other reagents are:

	Mg^{2+} (mM)	dNTPs (mM of each)	each primer (μM)	BSA (mg/mL)	Ta (°C)	no. initial cycles	no. indexing cycles
rbcL	2.5	0.2	0.2	0.5	56	25	20
16S	2.5	0.2	0.2	0.5	54	25	10
COI	2	0.2	0.2	0.5	51	40	10
indexing PCR	2	0.2	0.2	0.5	63	-	-

Figure C2. Histogram of identity matches to the best BLAST hit for all 432 prey taxa. Those with a match of 99% or more (the bar to the right, n = 261) are considered to be correctly identified to species level (see 4.3.4).

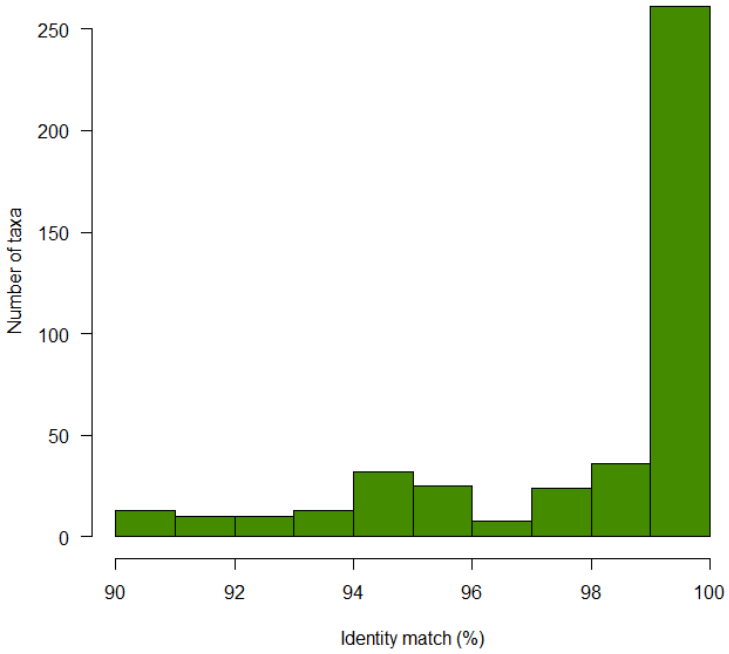

Figure C1 Correlation plot of pairwise comparisons across all rows within PCR plates (top panel) and all columns within PCR plates (bottom panel). The only systematically contaminated row or column was judged to be row 1.8 (towards left-hand side of top panel, mean r = 0.37).

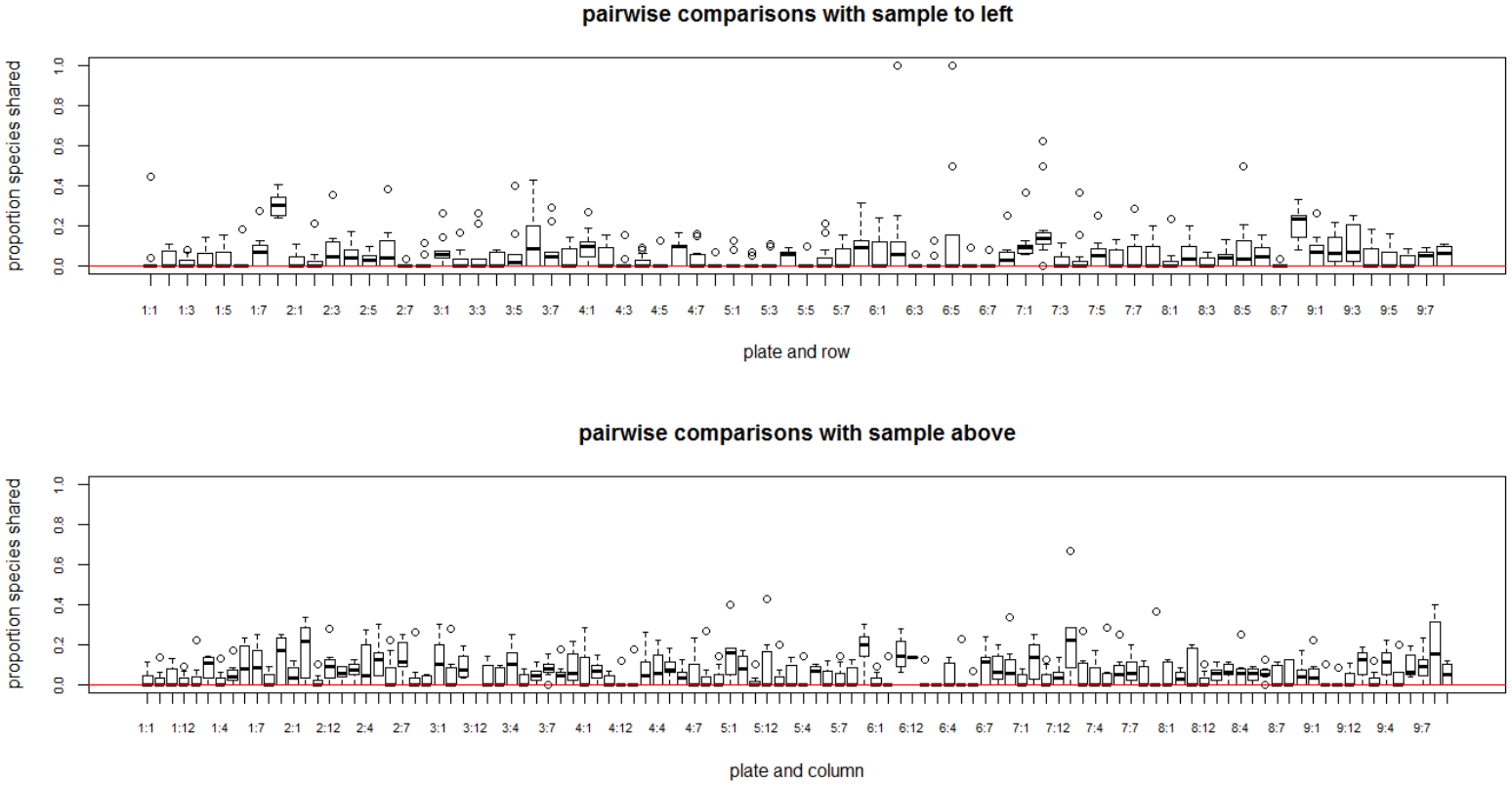

Table C1. Detailing all prey taxa (n = 432) identified in alphabetical taxonomic order. Identity refers to the match percentage with the best BLAST hit (see 4.3.4) and frequency to how many samples that taxa occurred in. Taxa shown with 0 frequency are those that were only found in replicate samples (n = 11).

Class	Order	Family	Genus	Species	Identity (%)	Frequency
Arachnida	*Araneae*	*Agelenidae*	*Tegenaria*	*Tegenaria rothi*	91	6
		Amaurobiidae	*Amaurobius*	*Amaurobius erberi*	92	4
			Callobius	*Callobius nomeus*	91	1
		Anyphaenidae	*Anyphaena*	*Anyphaena accentuata*	100	26
				Anyphaena aperta	90	45
		Araneidae	*Araneus*	*Araneus diadematus*	100	21
			Argiope	*Argiope trifasciata*	94	4
			Zygiella	*Zygiella atrica*	100	1
				Zygiella x-notata	100	1
		Clubionidae	*Clubiona*	*Clubiona littoralis*	91	4
				Clubiona moesta	92	1
				Clubiona norvegica	95	4
				Clubiona pallidula	100	0
		Dictynidae	*Emblyna*	*Emblyna annulipes*	91	1
		Linyphiidae	*Bathyphantes*	*Bathyphantes gracilis*	100	5
				Bathyphantes orica	92	27
				Bathyphantes pallidus	90	1
			Hypselistes	*Hypselistes florens*	95	1
			Lepthyphantes	*Lepthyphantes minutus*	99	4
			Microneta	*Microneta viaria*	100	1
			Neriene	*Neriene clathrata*	97	1
			Pityohyphantes	*Pityohyphantes costatus*	96	8

Arachnida	*Araneae*	*Linyphiidae*	*Porrhomma*	*Porrhomma convexum*	99	8
			Scironis	*Scironis sima*	90	1
				Scironis tarsalis	93	1
			Tenuiphantes	*Tenuiphantes zibus*	93	1
		Philodromidae	*Philodromus*	*Philodromus aureolus*	100	15
		Selenopidae	*Selenops*	*Selenops annulatus*	94	1
		Tetragnathidae	*Metellina*	*Metellina mengei*	100	7
			Tetragnatha	*Tetragnatha extensa*	100	1
		Theridiidae	*Enoplognatha*	*Enoplognatha ovata*	100	6
			Phylloneta	*Phylloneta impressa*	93	2
			Theridion	*Theridion varians*	99	6
	Opiliones	*Nemastomatidae*	*NA*	*Nemastomatidae sp.*	92	1
		Phalangiidae	*Oligolophus*	*Oligolophus tienmushanensis*	90	1
			Phalangium	*Phalangium opilio*	93	7
				Phalangiidae sp.	97	5
Clitellata	*Haplotaxida*	*Lumbricidae*	*Aporrectodea*	*Aporrectodea limicola*	99	1
			Lumbricus	*Lumbricus sp.*	100	2
				Lumbricidae sp.	95	3
		Tubificidae	*Nais*	*Nais sp.*	90	0
Collembola	*Collembola*	*Entomobryidae*	*Entomobrya*	*Entomobrya multifasciata*	99	1
				Entomobrya nivalis	100	104
				Entomobrya sp.	99	12
				Entomobrya unostrigata	100	1
			Lepidocyrtus	*Lepidocyrtus sp.*	90	1
			NA	*Entomobryidae sp.*	100	3
		Hypogastruridae	*Hypogastrura*	*Hypogastrura sp.*	99	1
		Isotomidae	*Isotoma*	*Isotoma viridis*	95	2

Collembola	*Collembola*	*Isotomidae*	*NA*	*Isotomidae sp.*	99	1
		Paronellidae	*NA*	*Paronellidae sp.*	94	3
		Tomoceridae	*Tomocerus*	*Tomocerus sp.*	100	2
Diplopoda	*Julida*	*Julidae*	*Cylindroiulus*	*Cylindroiulus latestriatus*	100	1
Gastropoda	*Stylommatophora*	*Agriolimacidae*	*Deroceras*	*Deroceras sp.*	100	11
		Arionidae	*Arion*	*Arion owenii*	100	2
				Arion sp.	100	1
		Hygromiidae	*Trochulus*	*Trochulus striolatus*	100	8
		Limacidae	*Ambigolimax*	*Ambigolimax valentianus*	92	1
			Lehmannia	*Lehmannia marginata*	100	1
Insecta	*Blattodea*	*Ectobiidae*	*Ectobius*	*Ectobius lapponicus*	92	5
				Ectobiidae sp.	93	1
	Coleoptera	*Anobiidae*	*Dryophilus*	*Dryophilus pusillus*	100	18
		Brentidae	*Betulapion*	*Betulapion simile*	100	15
			Exapion	*Exapion formaneki*	95	1
		Carabidae	*Amara*	*Amara aulica*	100	2
			Elaphropus	*Elaphropus parvulus*	94	23
			Loricera	*Loricera pilicornis*	100	1
			Nebria	*Nebria brevicollis*	99	4
			Poecilus	*Poecilus versicolor*	94	5
		Chrysomelidae	*Galerucella*	*Galerucella lineola*	100	1
			Gonioctena	*Gonioctena quinquepunctata*	93	1
			Longitarsus	*Longitarsus melanocephalus*	99	1
		Coccinellidae	*Harmonia*	*Harmonia axyridis*	100	1
		Curculionidae	*Andrion*	*Andrion regensteinense*	100	18
			Anthonomus	*Anthonomus ulmi*	99	26
			Coeliodes	*Coeliodes sp.*	99	7

Insecta	*Coleoptera*	*Curculionidae*	*Hypera*	*Hypera miles*	91	1
				Hypera rumicis	98	1
			Otiorhynchus	*Otiorhynchus singularis*	100	2
			Phloeotribus	*Phloeophthorus rhododactylus*	96	5
			Phyllobius	*Phyllobius calcaratus*	99	1
				Phyllobius pomaceus	94	1
				Phyllobius pyri	100	22
			Pityophthorus	*Pityophthorus pubescens*	99	4
			Polydrusus	*Polydrusus pilosus*	100	56
			Strophosoma	*Strophosoma capitatum*	100	1
				Strophosoma fulvicorne	91	0
			Tomicus	*Tomicus piniperda*	99	7
			Trypodendron	*Trypodendron domesticum*	100	5
			NA	*Curculionidae sp.*	99	3
		Derodontidae	*Laricobius*	*Laricobius erichsoni*	100	2
		Elateridae	*Melanotus*	*Melanotus castanipes*	100	5
		Helophoridae	*Helophorus*	*Helophorus aquaticus*	97	1
		Leiodidae	*Catops*	*Catops chrysomeloides*	100	1
		Nitidulidae	*Epuraea*	*Epuraea aestiva*	98	1
		Scarabaeidae	*Aphodius*	*Aphodius fasciatus*	99	1
				Aphodius prodromus	100	7
				Aphodius sphacelatus	99	1
		Scirtidae	*Contacyphon*	*Cyphon padi*	100	3
		Scraptiidae	*Anaspis*	*Anaspis maculata*	100	3
		Staphylinidae	*Philonthus*	*Philonthus decorus*	95	1
				Philonthus rotundicollis	100	1
			Phyllodrepoidea	*Phyllodrepoidea crenata*	99	4

Insecta	*Coleoptera*	*Staphylinidae*	*Tachinus*	*Tachinus subterraneus*	99	1
	Diptera	*Anisopodidae*	*Sylvicola*	*Sylvicola cinctus*	99	1
				Sylvicola sp.	99	1
		Anthomyzidae	*Anthomyza*	*Anthomyza anderssoni*	100	1
		Bibionidae	*Bibio*	*Bibio sp.*	97	1
			NA	*Bibionidae sp.*	100	3
		Calliphoridae	*Lucilia*	*Lucilia sericata*	99	1
			Pollenia	*Pollenia griseotomentosa*	99	1
				Pollenia labialis	100	3
				Pollenia pediculata	100	3
				Pollenia rudis	100	4
		Cecidomyiidae	*NA*	*Cecidomyiidae sp.*	97	89
			NA	*Cecidomyiinae sp.*	96	90
		Ceratopogonidae	*Forcipomyia*	*Forcipomyia nigrans*	99	1
				Forcipomyia tenuis	100	2
		Chironomidae	*Chaetocladius*	*Chaetocladius melaleucus*	99	4
				Chaetocladius sp.	100	1
			Cricotopus	*Cricotopus bicinctus*	95	9
				Cricotopus sp.	94	35
			Gymnometriocnemus	*Gymnometriocnemus brumalis*	100	1
				Gymnometriocnemus sp.	91	3
			Halocladius	*Halocladius variabilis*	100	1
			Limnophyes	*Limnophyes asquamatus*	100	1
				Limnophyes difficilis	100	13
				Limnophyes edwardsi	100	27
				Limnophyes pentaplastus	99	7
			Metriocnemus	*Metriocnemus albolineatus*	100	34

Insecta	*Diptera*	*Chironomidae*	*Metriocnemus*	*Metriocnemus eurynotus*	100	1
				Metriocnemus sp.	95	9
			Micropsectra	*Micropsectra pallidula*	99	1
			Nanocladius	*Nanocladius shigaensis*	96	29
			Orthocladius	*Orthocladius oliveri*	94	1
			Orthocladius	*Orthocladius sp.*	94	9
				Orthocladiinae sp.	96	19
			Paraphaenocladius	*Paraphaenocladius exagitans*	100	2
				Paraphaenocladius impensus	99	5
				Paraphaenocladius irritus	98	1
			Paratrichocladius	*Paratrichocladius sp.*	97	3
			Prodiamesa	*Prodiamesa olivacea*	100	1
			Rheocricotopus	*Rheocricotopus effusus*	100	4
				Rheocricotopus robacki	98	1
			Smittia	*Smittia sp.*	100	9
			Sympotthastia	*Sympotthastia sp.*	97	1
			Synorthocladius	*Synorthocladius semivirens*	95	5
			Tvetenia	*Tvetenia bavarica*	97	3
			NA	*Chironomidae sp.*	100	28
		Chloropidae	*Elachiptera*	*Elachiptera decipiens*	100	2
			Hapleginella	*Hapleginella conicola*	97	1
		Culicidae	*Culex*	*Culex pipiens*	100	4
			NA	*Culicidae sp.*	94	1
		Drosophilidae	*Drosophila*	*Drosophila rellima*	94	1
		Dryomyzidae	*Dryomyza*	*Dryomyza anilis*	100	2
		Ephydridae	*Philygria*	*Philygria vittipennis*	99	0
		Heleomyzidae	*Suillia*	*Suillia variegata*	99	26

Insecta	*Diptera*	*Heleomyzidae*	*Tephrochlamys*	*Tephrochlamys rufiventris*	97	100
		Muscidae	*Helina*	*Helina sp.*	91	3
		Mycetophilidae	*Aglaomyia*	*Aglaomyia gatineau*	95	1
			NA	*Mycetophilinae sp.*	96	1
		Opomyzidae	*Opomyza*	*Opomyza florum*	99	26
		Pallopteridae	*Palloptera*	*Palloptera ustulata*	95	2
		Psychodidae	*Pericoma*	*Pericoma sp.*	94	1
			Pneumia	*Pneumia borealis*	94	1
			Psychoda	*Psychoda sp.*	100	1
			NA	*Psychodidae sp.*	95	1
		Rhagionidae	*Rhagio*	*Rhagio lineola*	100	1
		Sarcophagidae	*Oxysarcodexia*	*Oxysarcodexia varia*	94	1
		Scathophagidae	*NA*	*Scathophagidae sp.*	96	4
		Scatopsidae	*Scatopse*	*Scatopse sp.*	96	2
		Sciaridae	*Claustropyga*	*Claustropyga abblanda*	99	3
			Corynoptera	*Corynoptera trepida*	100	3
			Ctenosciara	*Ctenosciara hyalipennis*	100	1
			Leptosciarella	*Leptosciarella viatica*	100	1
			NA	*Sciaridae sp.*	96	6
		Simuliidae	*Cnephia*	*Cnephia ornithophilia*	93	2
			Simulium	*Simulium intermedium*	100	1
		Sphaeroceridae	*Spelobia*	*Spelobia sp.*	96	12
			NA	*Sphaeroceridae sp.*	98	4
		Syrphidae	*Dasysyrphus*	*Dasysyrphus pinastri*	100	1
			Episyrphus	*Episyrphus balteatus*	100	1
			Eristalis	*Eristalis stipator*	98	2
				Eristalis tenax	100	1

Insecta	*Diptera*	*Syrphidae*	*Eupeodes*	*Eupeodes latifasciatus*	99	1
			Platycheirus	*Platycheirus sp.*	100	8
			Syritta	*Syritta pipiens*	99	1
			NA	*Syrphidae sp.*	100	4
		Tachinidae	*Exorista*	*Exorista rustica*	98	2
			Lypha	*Lypha ruficauda*	100	2
			Triarthria	*Triarthria sp.*	100	4
		Tachinidae	*NA*	*Tachinidae sp.*	99	2
	Ephemeroptera	*Baetidae*	*Baetis*	*Baetis sp.*	97	3
	Hemiptera	*Acanthosomatidae*	*Elasmostethus*	*Elasmostethus interstinctus*	100	1
		Adelgidae	*Adelges*	*Adelges abietis*	100	18
				Adelges lariciatus	99	2
				Adelges laricis	100	115
			Pineus	*Pineus orientalis*	100	3
				Pineus sp.	95	3
		Aphididae	*Aphis*	*Aphis fabae*	100	2
			Brachycaudus	*Brachycaudus helichrysi*	100	14
			Clethrobius	*Clethrobius comes*	98	1
			Drepanosiphum	*Drepanosiphum platanoidis*	100	126
			Elatobium	*Elatobium abietinum*	100	4
			Euceraphis	*Euceraphis betulae*	100	171
				Euceraphis borealis	98	5
				Euceraphis punctipennis	100	56
				Euceraphis sp.	97	2
			Macrosiphoniella	*Macrosiphoniella millefolii*	100	1
			Macrosiphum	*Macrosiphum euphorbiae*	100	1
			Periphyllus	*Periphyllus acericola*	99	1

Insecta	*Hemiptera*	*Aphididae*	*Pterocomma*	*Pterocomma pilosum*	100	2
			Rhopalosiphum	*Rhopalosiphum insertum*	100	39
				Rhopalosiphum padi	100	26
			Sitobion	*Sitobion fragariae*	99	5
		Cicadellidae	*Empoasca*	*Empoasca decipiens*	98	5
			Eupteryx	*Eupteryx atropunctata*	98	2
			Oncopsis	*Oncopsis monticola*	99	3
				Oncopsis tristis	100	1
		Greenideidae	*Greenidea*	*Greenidea longirostrum*	94	2
		Lachnidae	*Cinara*	*Cinara laricis*	100	1
			Eulachnus	*Eulachnus piniarmandifoliae*	94	1
		Miridae	*Harpocera*	*Harpocera thoracica*	100	1
			Pinalitus	*Pinalitus viscicola*	95	2
			Psallus	*Psallus ambiguus*	98	1
				Psallus varians	94	1
			Rhabdomiris	*Rhabdomiris striatellus*	94	1
			Stenodema	*Stenodema calcarata*	99	2
				Stenodema holsata	100	1
		Pentatomidae	*Palomena*	*Palomena prasina*	100	2
			Pentatoma	*Pentatoma rufipes*	100	23
		Reduviidae	*Empicoris*	*Empicoris vagabundus*	99	1
	Hymenoptera	*Apidae*	*Apis*	*Apis mellifera*	100	1
			Bombus	*Bombus lucorum*	100	1
				Bombus terrestris	100	1
		Argidae	*Schizocerella*	*Schizocerella pilicornis*	92	2
		Braconidae	*Apanteles*	*Apanteles carpatus*	100	1
				Apanteles sp.	99	1

Insecta	*Hymenoptera*	*Braconidae*	*Cheloninae*	*Cheloninae gen.*	95	1
			Dolichogenidea	*Dolichogenidea absona*	99	20
				Dolichogenidea sp.	99	23
			Ephedrus	*Ephedrus plagiator*	100	0
			Meteorus	*Meteorus jaculator*	100	1
				Meteorus pendulus	99	4
				Meteorus sp.	100	1
			Pygostolus	*Pygostolus sticticus*	99	1
			Stantonia	*Stantonia sp.*	98	1
			NA	*Braconidae sp.*	94	10
			NA	*Microgastrinae sp.*	95	1
			NA	*Orgilinae sp.*	91	3
		Colletidae	*Colletes*	*Colletes daviesanus*	100	1
		Cynipidae	*Andricus*	*Andricus coriarius*	97	9
				Andricus curvator	98	5
				Andricus kollari	99	38
				Andricus quercustozae	99	43
			Cynips	*Cynips quercus*	95	27
				Cynips quercusfolii	98	4
			Neuroterus	*Neuroterus numismalis*	100	5
			Neuroterus	*Neuroterus quercusbaccarum*	100	41
			Rhoophilus	*Rhoophilus loewi*	93	3
			Trigonaspis	*Trigonaspis mendesi*	97	20
			NA	*Cynipidae sp.*	97	17
		Eulophidae	*Aprostocetus*	*Aprostocetus sp.*	92	1
			Baryscapus	*Baryscapus sp.*	95	1
		Figitidae	*Alloxysta*	*Alloxysta semiaperta*	94	1

Insecta	*Hymenoptera*	*Formicidae*	*Azteca*	*Azteca beltii*	91	2
				Azteca sp.	90	1
		Ichneumonidae	*Aperileptus*	*Aperileptus sp.*	96	1
			Diadegma	*Diadegma majale*	99	2
				Diadegma sp.	98	1
			Dusona	*Dusona ellopiae*	95	2
			Glypta	*Glypta arctica*	97	10
			Hyposoter	*Hyposoter horticola*	94	1
			Lathrostizus	*Lathrostizus forticanda*	100	1
			Rhimphoctona	*Rhimphoctona longicauda*	94	1
		Ichneumonidae	*Scambus*	*Scambus calobatus*	100	1
				Scambus sp.	97	1
				Scambus vesicarius	99	1
			NA	*Campopleginae sp.*	99	19
			NA	*Ichneumonidae sp.*	99	8
			NA	*Orthocentrinae sp.*	96	1
			NA	*Pimplinae sp.*	96	2
		Platygastridae	*NA*	*Platygastridae sp.*	98	9
		Pteromalidae	*Pteromalus*	*Pteromalus dolichurus*	99	1
				Pteromalidae sp.	91	1
		Tenthredinidae	*Empria*	*Empria sp.*	93	2
			Periclista	*Periclista albida*	99	1
		Torymidae	*NA*	*Torymidae sp.*	95	2
		Vespidae	*Dolichovespula*	*Dolichovespula saxonica*	100	1
		Xyelidae	*Xyela*	*Xyela minor*	100	2
		NA	*NA*	*Hymenoptera sp.*	98	195
	Lepidoptera	*Adelidae*	*Adela*	*Adela cuprella*	95	1

Insecta	*Lepidoptera*	*Agonoxenidae*	*Chrysoclista*	*Chrysoclista lathamella*	97	1
		Argyresthiidae	*Argyresthia*	*Argyresthia albistria*	100	7
				Argyresthia brockeella	99	71
				Argyresthia glabratella	100	2
				Argyresthia goedartella	100	268
				Argyresthia laevigatella	100	31
		Batrachedridae	*Batrachedra*	*Batrachedra praeangusta*	100	3
		Blastobasidae	*Blastobasis*	*Blastobasis maroccanella*	99	10
		Coleophoridae	*Coleophora*	*Coleophora flavipennella*	100	5
				Coleophora laricella	100	33
				Coleophora orbitella	100	1
			Coleophora	*Coleophora sp.*	100	28
		Crambidae	*Eudonia*	*Eudonia lacustrata*	100	3
				Eudonia mercurella	100	2
			NA	*Crambidae sp.*	99	2
		Drepanidae	*Achlya*	*Achlya flavicornis*	100	8
		Elachistidae	*Agonopterix*	*Agonopterix assimilella*	99	11
		Elachistidae	*Depressaria*	*Depressaria silesiaca*	99	1
			Semioscopis	*Semioscopis avellanella*	100	9
				Semioscopis steinkellneriana	98	1
		Erebidae	*Hypena*	*Hypena proboscidalis*	100	2
			Palthis	*Palthis sp.*	97	1
			Phragmatobia	*Phragmatobia fuliginosa*	100	1
		Gelechiidae	*Carpatolechia*	*Carpatolechia fugitivella*	100	10
			Exoteleia	*Exoteleia dodecella*	98	4
			Teleiodes	*Teleiodes luculella*	100	1
		Geometridae	*Agriopis*	*Agriopis aurantiaria*	100	2

Insecta	*Lepidoptera*	*Geometridae*	*Agriopis*	*Agriopis marginaria*	100	3
			Alcis	*Alcis repandata*	100	1
			Alsophila	*Alsophila aescularia*	100	19
			Apocheima	*Apocheima pilosaria*	100	1
			Archiearis	*Archiearis parthenias*	98	2
			Campaea	*Campaea margaritaria*	99	15
			Cleorodes	*Cleorodes lichenaria*	100	3
			Colotois	*Colotois pennaria*	100	6
			Deileptenia	*Deileptenia ribeata*	100	34
			Dysstroma	*Dysstroma sp.*	95	0
			Epirrita	*Epirrita autumnata*	100	2
				Epirrita christyi	100	3
			Erannis	*Erannis defoliaria*	99	1
			Eupithecia	*Eupithecia abbreviata*	100	1
				Eupithecia tenuiata	100	16
			Geometra	*Geometra papilionaria*	100	15
			Hydriomena	*Hydriomena furcata*	100	3
				Hydriomena impluviata	100	2
				Hydriomena ruberata	99	1
			Hylaea	*Hylaea fasciaria*	100	4
			Operophtera	*Operophtera brumata*	100	27
				Operophtera fagata	99	16
			Opisthograptis	*Opisthograptis luteolata*	100	3
			Pasiphila	*Pasiphila sp.*	100	4
			Scopula	*Scopula sp.*	93	3
			Trichopteryx	*Trichopteryx carpinata*	100	0
		Gracillariidae	*Phyllonorycter*	*Phyllonorycter lautella*	99	2

Insecta	*Lepidoptera*	*Gracillariidae*	*Phyllonorycter*	*Phyllonorycter quercifoliella*	100	0
				Phyllonorycter roboris	100	3
			NA	*Gracillariidae sp.*	95	1
		Heliodinidae	*Embola*	*Heliodines ionis*	95	8
		Hesperiidae	*Phanus*	*Phanus sp.*	100	1
		Lymantriidae	*Euproctis*	*Euproctis similis*	100	1
		Lyonetiidae	*Leucoptera*	*Leucoptera spartifoliella*	100	11
		Mimallonidae	*Mimallonidae*	*Mimallonidae sp.*	94	1
		Nepticulidae	*Stigmella*	*Stigmella sp.*	100	1
		Noctuidae	*Acronicta*	*Acronicta leporina*	100	1
			Agrochola	*Agrochola circellaris*	100	26
				Agrochola lychnidis	97	1
				Agrochola macilenta	99	6
			Allophyes	*Allophyes oxyacanthae*	100	3
			Anaplectoides	*Anaplectoides prasina*	100	1
			Apamea	*Apamea crenata*	100	1
				Apamea remissa	100	2
			Atethmia	*Atethmia centrago*	100	6
			Brachylomia	*Brachylomia discinigra*	95	3
			Caradrina	*Caradrina morpheus*	99	1
			Chilodes	*Chilodes maritimus*	100	6
			Conistra	*Conistra vaccinii*	100	5
			Cosmia	*Cosmia trapezina*	100	3
			Eupsilia	*Eupsilia transversa*	100	4
			Graphiphora	*Graphiphora augur*	100	1
			Hillia	*Hillia iris*	97	2
			Noctua	*Noctua fimbriata*	100	1

Insecta	*Lepidoptera*	*Noctuidae*	*Orthosia*	*Orthosia cerasi*	100	1
				Orthosia gothica	100	1
			Panolis	*Panolis flammea*	100	5
			Sideridis	*Sideridis congermana*	98	1
			Tiliacea	*Tiliacea citrago*	100	0
			Xanthia	*Xanthia icteritia*	100	9
				Xanthia togata	99	4
		Nolidae	*Pseudoips*	*Pseudoips prasinanus*	100	1
		Notodontidae	*Drymonia*	*Drymonia dodonaea*	95	1
			Furcula	*Furcula bicuspis*	99	1
		Oecophoridae	*Barea*	*Barea discincta*	95	1
				Barea eclecta	98	3
			Diurnea	*Diurnea fagella*	99	3
			Endrosis	*Endrosis sarcitrella*	100	8
			Hofmannophila	*Hofmannophila pseudospretella*	100	3
		Prodoxidae	*Lampronia*	*Lampronia corticella*	99	1
		Pyralidae	*Cryptoblabes*	*Cryptoblabes bistriga*	100	1
		Tineidae	*Monopis*	*Monopis laevigella*	100	4
		Tischeriidae	*Tischeria*	*Tischeria ekebladella*	99	1
		Tortricidae	*Acleris*	*Acleris abietana*	98	1
				Acleris sp.	98	3
			Anacrusis	*Anacrusis sp.*	99	1
			Apotomis	*Apotomis capreana*	99	6
				Apotomis sp.	95	0
			Cochylis	*Cochylis nana*	100	1
			Dichelia	*Dichelia histrionana*	100	1
			Ditula	*Ditula angustiorana*	99	8

Insecta	*Lepidoptera*	*Tortricidae*	*Epinotia*	*Epinotia bilunana*	100	65
				Epinotia immundana	99	8
				Epinotia pygmaeana	98	0
				Epinotia ramella	100	165
				Epinotia sp.	99	105
				Epinotia tedella	100	3
			Notocelia	*Notocelia cynosbatella*	99	5
				Notocelia trimaculana	100	2
			Pammene	*Pammene regiana*	100	2
			Pandemis	*Pandemis cerasana*	100	6
				Pandemis cinnamomeana	100	7
				Pandemis heparana	100	1
			Ptycholoma	*Ptycholoma lecheana*	100	3
			Rhyacionia	*Rhyacionia pinivorana*	100	2
			Spilonota	*Spilonota laricana*	100	11
		Yponomeutidae	*Cedestis*	*Cedestis subfasciella*	99	5
			Ocnerostoma	*Ocnerostoma friesei*	100	3
				Ocnerostoma piniariella	99	7
			Prays	*Prays fraxinella*	99	3
				Prays ruficeps	100	7
		Ypsolophidae	*Ypsolopha*	*Ypsolopha ustella*	100	5
	Neuroptera	*Chrysopidae*	*Nothochrysa*	*Nothochrysa capitata*	100	4
		Coniopterygidae	*Coniopteryx*	*Coniopteryx tineiformis*	100	6
		Hemerobiidae	*Wesmaelius*	*Wesmaelius nervosus*	100	9
	Plecoptera	*Chloroperlidae*	*Siphonoperla*	*Siphonoperla torrentium*	98	1
	Psocoptera	*Caeciliusidae*	*Valenzuela*	*Valenzuela flavidus*	100	4
		NA	*NA*	*Psocoptera sp.*	94	3

Insecta	*Thysanoptera*	*Thripidae*	*Taeniothrips*	*Taeniothrips inconsequens*	100	1
			NA	*Thripinae sp.*	90	1
Malacostraca	*Isopoda*	*Armadillidiidae*	*Armadillidium*	*Armadillidium vulgare*	98	12

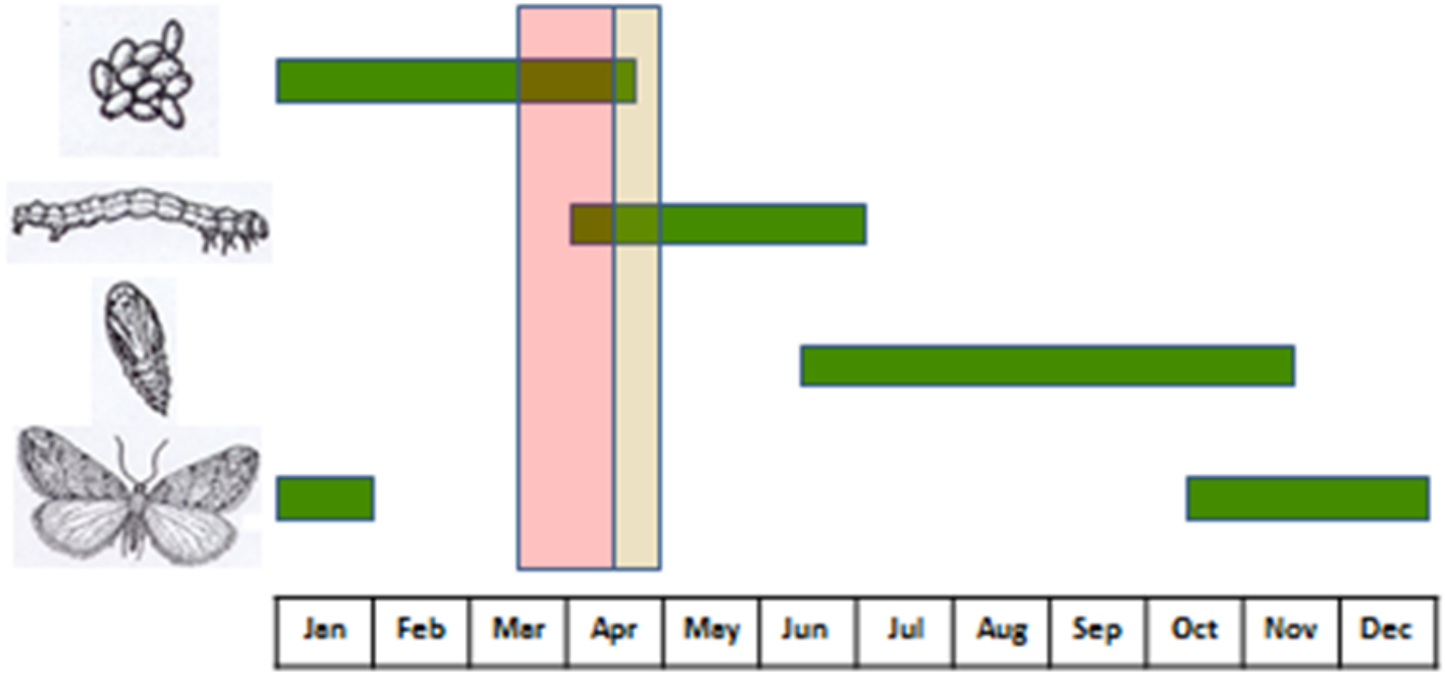

Figure C3 Life-cycle phenology of winter moth, with phenological data taken from Waring & Townsend (2017) and ukmoths.co.uk. Transparent yellow bar represents the approximate window winter moth is found in the diet of adult blue tits in this study whilst the transparent red bar represents the approximate window that they were not found, highlighting the probability of this occurrence being attributable to early instar larvae (caterpillars).

Appendix D

Supplementary material for Chapter 5

Table D1. All identified caterpillar species collected along the transect, with host tree taxa, sites collected at and overall total of identified specimens, in taxonomic order. Tree genera codes used: AL Alder, AS Ash, BE Beech, BI Birch, CH Cherry, EL Elm, HA Hazel, OK Oak, RO Rowan, SY Sycamore, WL Willow. Site codes (including respective latitudes and elevations etc.) can be found in Table 2.1.

Species	English Name	Trees	Sites	Total
		Coleoptera		
Gonioctena pallida	Leaf Beetle sp.	WL (6)	CAL (6)	6
		Diptera		
Syrphus ribesii	Common Banded Hoverfly	BI (1), OK (2), SY (4), WL (1)	BAD (1), FOF (2), MCH (1), PTH (1), SER (3)	8
Syrphus torvus	Hairy-eyed Hoverfly	BI (1), SY (1)	FOU (1), LVN (1)	2
Parasyrphus punctulatus	Hoverfly sp.	SY (1)	MCH (1)	1
		Lepidoptera: Lasiocampidae		
Poecilocampa populi	December Moth	SY (1)	STY (1)	1
		Lepidoptera: Geometridae		
Alsophila aescularia	March Moth	AL (1), OK (1)	DOR (1), FOU (1)	2
Hydriomena furcata	July Highflyer	WL (3)	DNS (2), SER (1)	3
Epirrita dilutata	November Moth	OK (1)	SPD (1)	1
Epirrita christyi	Pale November Moth	BE (3), BI (4), EL (1), HA (1), SY (3)	AVN (1), BIR (1), BLA (1), DEL (1), DUN (2), FOF (1), KCK (3), STY (1), TAI (1)	12
Epirrita autumnata	Autumnal Moth	AL (1), BI (9)	AVI (4), AVN (1), CAL (1), DNC (1), DOR (1), INS (1), MUN (1)	10
Epirrita filigrammaria	Small Autumnal Moth	BI (3), WL (2)	DLW (2), FSH (1), SLS (1), TOM (1)	5
Operophtera brumata	Winter Moth	AL (1), AS (1), BE (2), BI (38), CH (2), EL (2), HA (1), OK (29), RO (1), SY (11), WL (68)	AVI (6), AVN (5), BAD (1), BLA (2), BLG (5), CAL (3), DEL (2), DLW (6), DNC (68), DNM (1), DNS (2), DOR (1), EDI (2), FOF (1), FOU (4), FSH (2), GLF (14), INS (3), KCK (1), LVN (3), MCH (1), MUN (2), PTH (2), RSY (2), RTH (2), SER (4), SLS (2), SPD (5), STY (3), TOM (1)	156
Operophtera fagata	Northern Winter Moth	BI (26), RO (1)	AVI (11), BIR (3), CAL (1), DNC (9), FSH (1), INS (1), STY (1)	27
Eupithecia abbreviata	Brindled Pug	HA (1), OK (1)	GLF (1), SER (1)	2
Colotois pennaria	Feathered Thorn	BI (1), OK (2)	ART (1), AVI (1), FOU (1)	3

Phigalia pilosaria	Pale Brindled Beauty	AL (1), BI (7), OK (4), WL (1)	AVI (2), AVN (1), CAL (1), DAV (1), DNM (1), FSH (3), RTH (2), SPD (1), TOM (1)	13
Lycia hirtaria	Brindled Beauty	HA (1)	BLA (1)	1
Biston strataria	Oak Beauty	OK (2)	KCZ (2)	2
Agriopis leucophaearia	Spring Usher	OK (2)	KCZ (2)	2
Agriopis aurantiaria	Scarce Umber	BI (56), OK (2), RO (1), SY (1), WL (7)	ALN (2), AVI (7), AVN (2), CAL (6), CAR (4), CRU (2), DLW (3), DNC (9), FSH (6), INS (4), KCK (1), NEW (4), SLS (13), SPD (1), TOM (3)	67
Agriopis marginaria	Dotted Border	AL (1), BE (1), BI (12), SY (2)	ALN (2), AVI (2), BIR (3), DUN (1), INS (2), LVN (1), MCH (2), MUN (1), NEW (1), SLS (1)	16
Erannis defoliaria	Mottled Umber	BI (9), EL (1), OK (4), SY (2), WL (1)	ART (1), AVI (1), AVN (2), BLG (2), CAR (1), DNM (1), FOF (1), INS (4), LVN (1), RTH (1), SPD (2)	17
Deileptenia ribeata	Satin Beauty	OK (1)	MUN (1)	1
Alcis repandata	Mottled Beauty	BE (1)	MUN (1)	1
Ectropis crepuscularia	Engrailed	BI (1), SY (1)	BIR (1), INS (1)	2
Campaea margaritata	Light Emerald	BE (1), BI (2), RO (1)	ALN (1), AVI (1), DNC (1), MUN (1)	4
		Lepidoptera: Noctuidae		
Orthosia cerasi	Common Quaker	BE (1), BI (4), OK (8), RO (1), WL (2)	ART (1), AVI (1), AVN (1), BAD (1), BLA (1), CRU (1), DLW (2), DUN (1), INS (1), KCZ (2), MCH (1), SER (2), STY (1)	16
Orthosia gothica	Hebrew Character	OK (2), SY (1)	AVN (2), STY (1)	3
Orthosia incerta	Clouded Drab	BI (4), WL (2)	DNS (2), INS (2), LVN (1), SLS (1)	6
Anorthoa munda	Twin-spotted Quaker	HA (1)	GLF (1)	1
Brachylomia viminalis	Minor Shoulder-knot	WL (4)	DLW (1), DNC (3)	4
Allophyes oxyacanthae	Green-brindled Crescent	RO (1)	CAL (1)	1
Eupsilia transversa	The Satellite	EL (2), OK(2), SY (2)	BLG (3), FOF (1), FOU (1), MCH (1)	6
Conistra vaccinii	The Chestnut	BI (2), EL (1), HA (1), OK (8), SY (2), WL (1)	ART (1), AVN (1), BLG (1), CAL (1), EDI (1), FOU (1), GLF (2), MCH (1), MUN (1), NEW (1), RSY (1), RTH (1), SPD (2)	15
Agrochola circellaris	The Brick	BI (1)	NEW (1)	1
Cosmia trapezina	Dun-bar	HA (1), OK (1), SY (1)	GLF (1), KCK (1), KCZ (1)	3
		Lepidoptera: Ypsolophidae		

Ypsolopha parenthesella	White-shouldered Smudge	BI (2), WL (1)	DLW (1), DNC (2)	3
Ypsolopha ustella	Variable Smudge	BE (2), BI (5), OK (11), SY (1)	AVI (1), AVN (5), BLG (1), FSH (2), KCK (1), KCZ (2), MUN (4), SPD (3)	19
Ypsolopha sequella	Pied Smudge	SY (1)	RSY (1)	1
		Lepidoptera: Elachistidae		
Agonopterix ocellana	Red-letter Flat-body	WL (1)	SER (1)	1
		Lepidoptera: Torticidae		
Acleris sparsana	Ashy Button	SY (1)	BLG (1)	1
Tortricodes alternella	Winter Shade	OK (2)	GLF (2)	2
Ptycholoma lecheana	Brindled Tortrix	OK (2), SY (1)	BLG (1), FOF (2)	3
Pandemis cerasana	Barred Fruit-tree Tortrix	OK (5)	MCH (2), RTH (1), SPD (2)	5
Epinotia nisella	Grey Poplar Bell	WL (1)	SER (1)	1
Epinotia tenerana	Nut Bud Moth	AL (1)	DNM (1)	1
Epinotia cruciana	Willow Tortrix	BI (1), WL (1)	DLW (2)	2
Epinotia brunnichana	Large Birch Bell	BE (1)	STY (1)	1
		Lepidoptera: Crambidae		
Udea prunalis	Dusky Pearl	BE (1)	KCK (1)	1
		Hymenoptera		
Amauronematus sagmarius	Sawfly sp.	WL (1)	SER (1)	1
Amauronematus miltonotus	Sawfly sp.	WL (1)	SER (1)	1
Amauronematus humeralis	Sawfly sp.	WL (2)	MUN (2)	2
Amauronematus stenogaster	Sawfly sp.	WL (1)	DNM (1)	1
Amauronematus toeniatus	Sawfly sp.	BI (1)	AVN (1)	1
Amauronematus histrio	Sawfly sp.	WL (1)	DLW (1)	1
Amauronematus poppi	Sawfly sp.	BI (1)	DNM (1)	1
Amauronematus sp.	Sawfly sp.	WL (1)	STY (1)	1
Mesoneura opaca	Sawfly sp.	OK (2)	KCZ (1), SER (1)	2
Pamphilius sp.	Sawfly sp.	OK (1)	GLF (1)	1
Periclista lineolata	Sawfly sp.	OK (1)	MCH (1)	1
Periclista albida	Sawfly sp.	OK (1)	GLF (1)	1
Aleoides gastritor	Sawfly sp.	OK (1)	SPD (1)	1

Section D1 Analysis of caterpillar peak breadth

Aim: To understand whether the cut-off used (50% of peak height) for comparing caterpillar peak breadths in chapter 5 had any effect on the inferences gained.

Methods: First, five different quadratic curves were generated, representing different caterpillar temporal distributions (Fig D4). Then, the breadth of each peak was determined at 50% peak height cut-off and 75% peak height cut-off and the two peak breadth values for each quadratic curve plotted and compared. Finally, each curve was converted to the proportion scale, the breadths of each of these peaks identified at 50% and 75% peak height cut-offs and the two breadths for each plotted and compared.

Results: A linear relationship between the two peak breadth cut-offs was upheld for both the nominal and proportion scales (Fig D5).

Discussion: As the relationship between the two peak breadth cut-offs is linear, inferences made for, and comparisons generated between, caterpillar peak breadths will be similar regardless of the breadth cut-off utilised. Therefore, the nominate peak breadth cut-off analysed in chapter 5 (50% of total peak) had no bearing on the inferences gained from comparing caterpillar peak breadths.

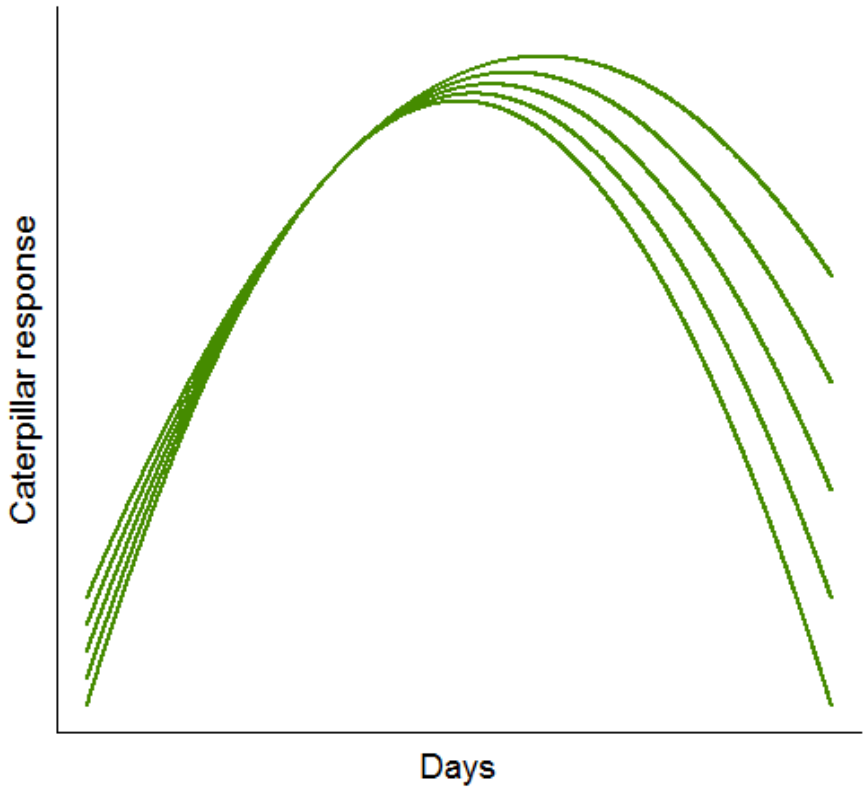

Figure D4 Illustrating the five different quadratic curves generated to represent different caterpillar peak temporal distributions.

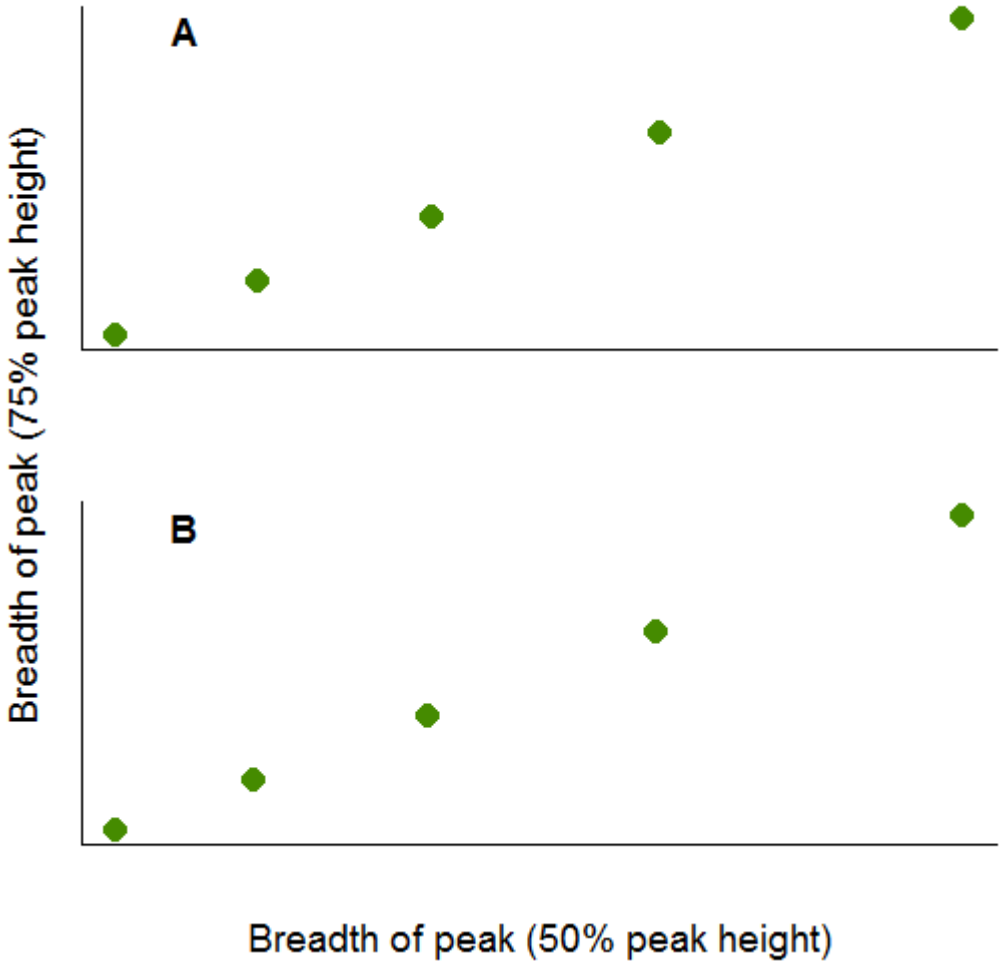

Figure D5 Linear relationship between two different peak breadth cut-offs (50% and 75%) on **A** the nominal scale and **B** the proportion scale, highlighting how the peak breadth cut-off used does not affect the comparison inferences gained due to the linear relationship.

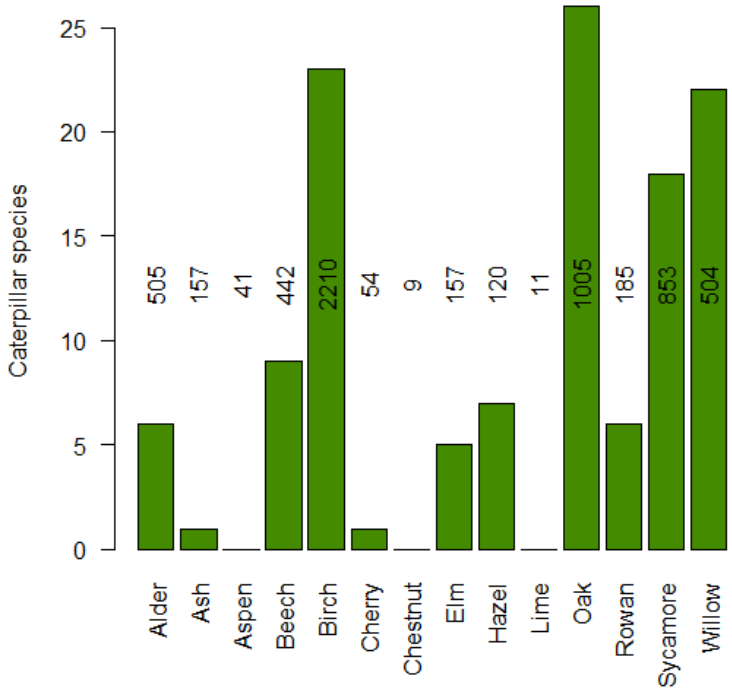

Fig D6 Caterpillar species found on each tree species, with vertical numbers representing the number of branch beats (sampling effort) that were performed on each tree species.

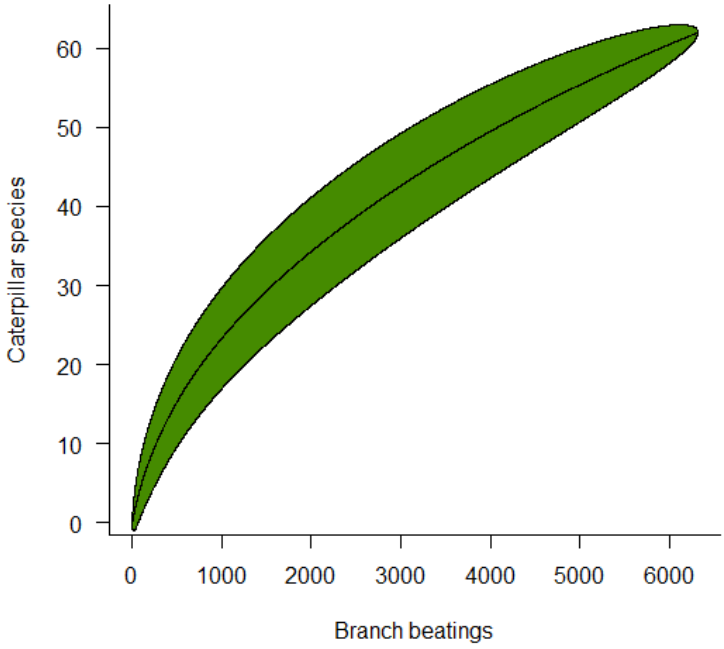

Fig D7 Estimated caterpillar species accumulation curve (mean ± se) for the transect. Estimated in the R package 'vegan' (Oksanen *et al* 2012).

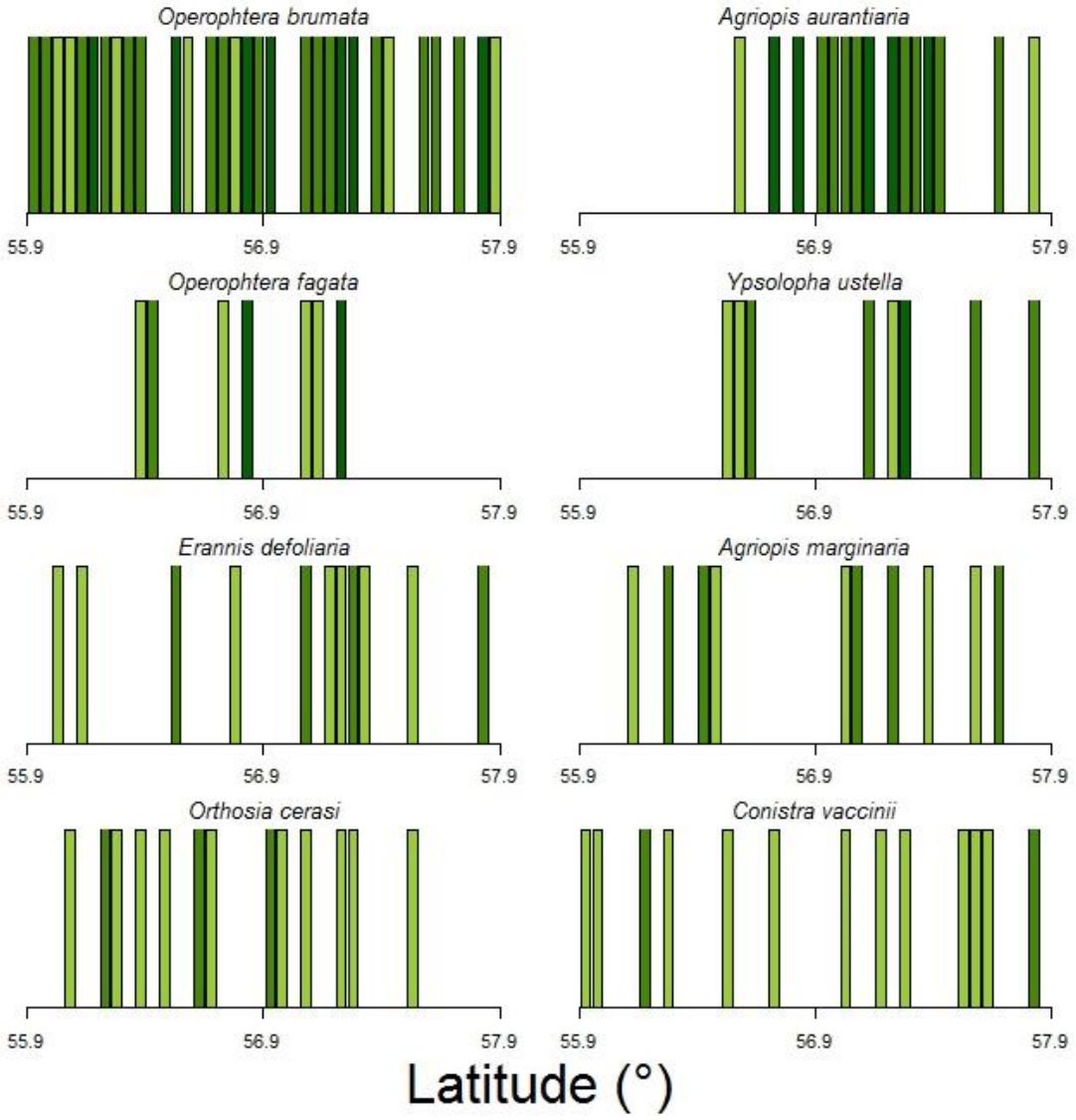

Figure D1 Latitudinal presence/absence of the eight most abundantly identified caterpillar species, with bars left to right representing sites from south to north. Empty bars signify no individuals identified at that site, with light green indicating one sampled individual, mid green two to four and dark green five plus individuals.

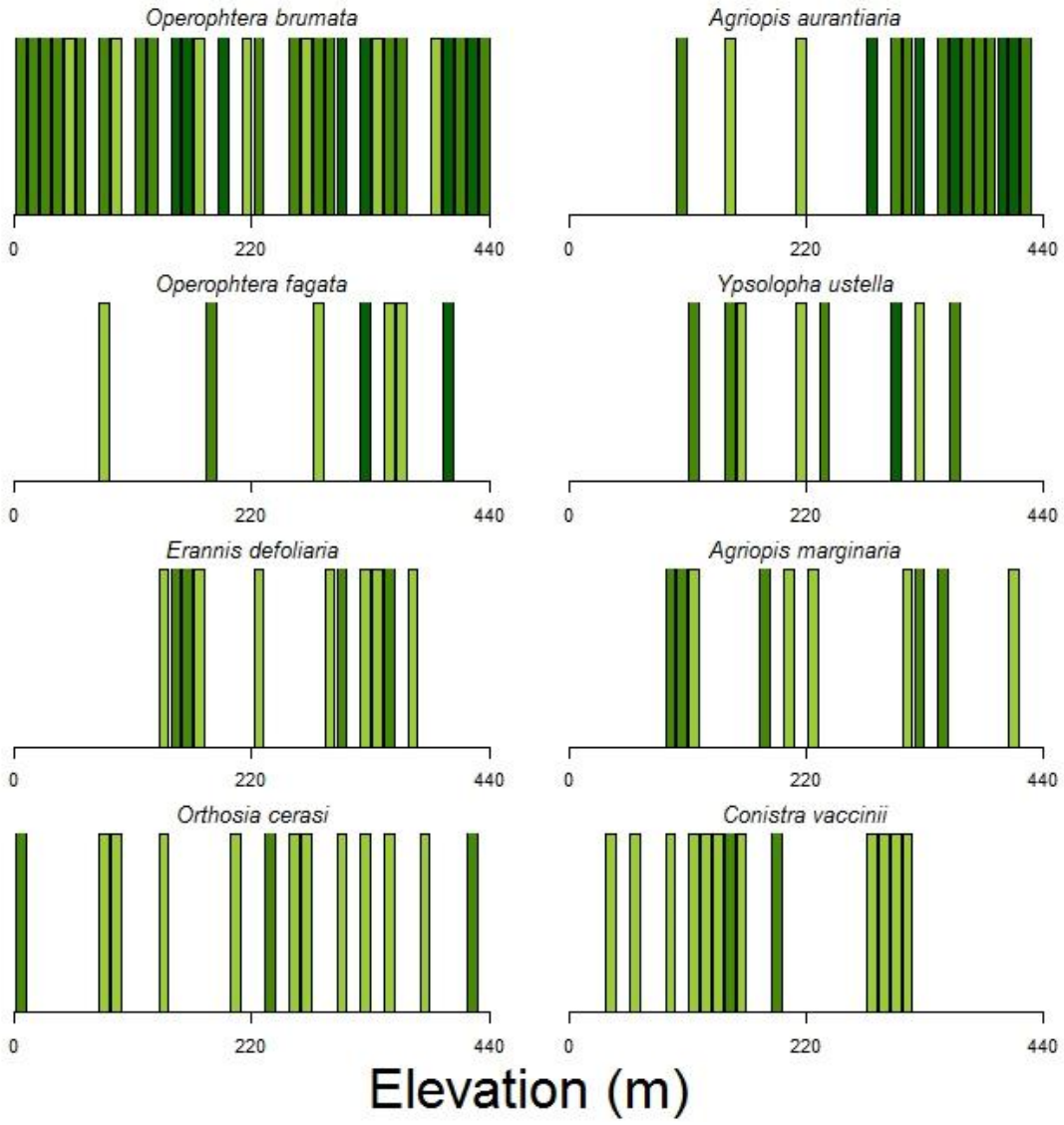

Figure D2 Elevational presence/absence of the eight most abundantly identified caterpillar species, with bars left to right representing sites from south to north. Empty bars signify no individuals identified at that site, with light green indicating one sampled individual, mid green two to four and dark green five plus individuals.

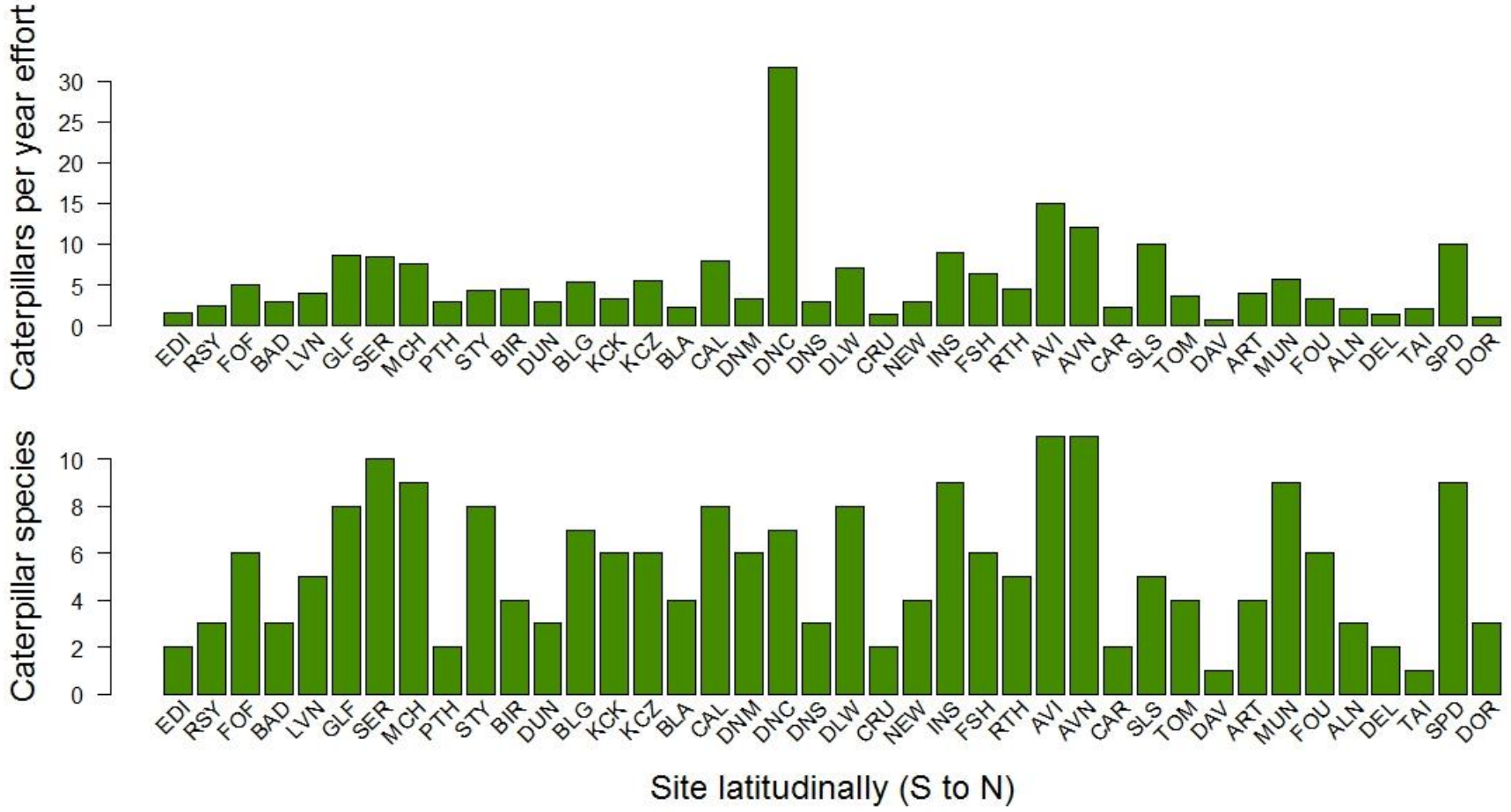

Figure D3 Top panel depicting caterpillars collected per year effort at each site. Bottom panel illustrates caterpillar species richness at each site. Both plots go from south to north from left to right (Table 2.1).

Appendix E

Statistical power analysis of the transect method

Statistical power analysis of the transect method

Introduction

In order to evaluate the statistical power of the transect and determine the probability of detecting an effect of a given size where there is one present under the inherent sample size constraints, I conducted a simulation-based power analysis looking at the relationship between temperature and blue tit phenology across sites. The aim was to explore the effects of increasing or decreasing the number of sites studied and the number of nestboxes operated per site to infer whether the transect used, incorporating six nestboxes each at forty sites, provides good statistical power.

Methods

The simulation was initially set up as per the real transect, with 40 sites and 240 total nestboxes. All variances and probabilities used were approximate reflections of real data, with nestbox occupancy set at 0.5 (see Table 2.3 and Appendix F), between-site temperature variance set at 0.7°C (see Table 3.2 and Figure B1), between-site phenological variance set at 10 days (see Table 3.2) and within-site phenological variance set at 30 days (see Table 3.2). Nestboxes 'occupied' per site were taken from a random binomial distribution based on the designated nestbox occupancy rate. For each site, a temperature record was created from a random normal distribution (mean = 10°C, sd = √ (between-site temperature variance)), and assigned to each 'occupied' nestbox within that site. Phenological site variance independent of the temperature variance was generated from a normal distribution (mean = 0, sd = √ (between-site phenological variance)). Finally, a phenological record was created for each 'occupied' nestbox by multiplying the temperature record by -4 (approximate real detected response slope of phenology to temperature, see Table 3.2) and adding this to the value obtained from a random normal distribution (mean = 0, sd = √ (within-site phenological variance) + independent phenological site variance). I then constructed a GLMM (Bates *et al.* 2015) with phenological record as the response variable, temperature record as a predictor variable, and site as a random variable, alongside an identical null model containing no predictor variable. A p-value representing the significance of temperature to predict phenology in that particular simulation was obtained by an ANOVA between the full and null models. 1000 simulations were run, with the p-values of each simulation stored, and the final statistical power inferred by recording the probability that across the simulations $p < 0.05$.

To test how statistical power varied depending on the number of sites studied and the number of nestboxes operated per site, I altered the starting parameters of the simulations in the following three ways:

i. I varied the number of sites studied from 5-80 (in breaks of five sites 5-40 and ten sites 40-80) but maintained the total number of nestboxes operated at 240, split evenly between sites.
ii. I varied the number of sites in a similar fashion to (i), but kept the number of nestboxes per site constant at six.
iii. I kept the number of sites constant at 40, but varied the number of nestboxes operated per site (2, 4, 8, with 6 already analysed in (i) and (ii)).

Results

Forty sites with six nestboxes per site provided excellent statistical power of 0.989 for detecting how temperature affects blue tit phenology (Figure E1). Statistical power reduces considerably when fewer than 35 sites are studied with six nestboxes per site, when fewer than 30 sites are studied when 240 nestboxes are distributed evenly across sites, and when only two or four nestboxes are operated at 40 sites (Figure E1). Statistical power to detect the effect of temperature on blue tit phenology does not increase very much with more sites studied, or with more nestboxes operated at 40 sites, rather plateauing at 40 sites with six nestboxes each (Figure E1).

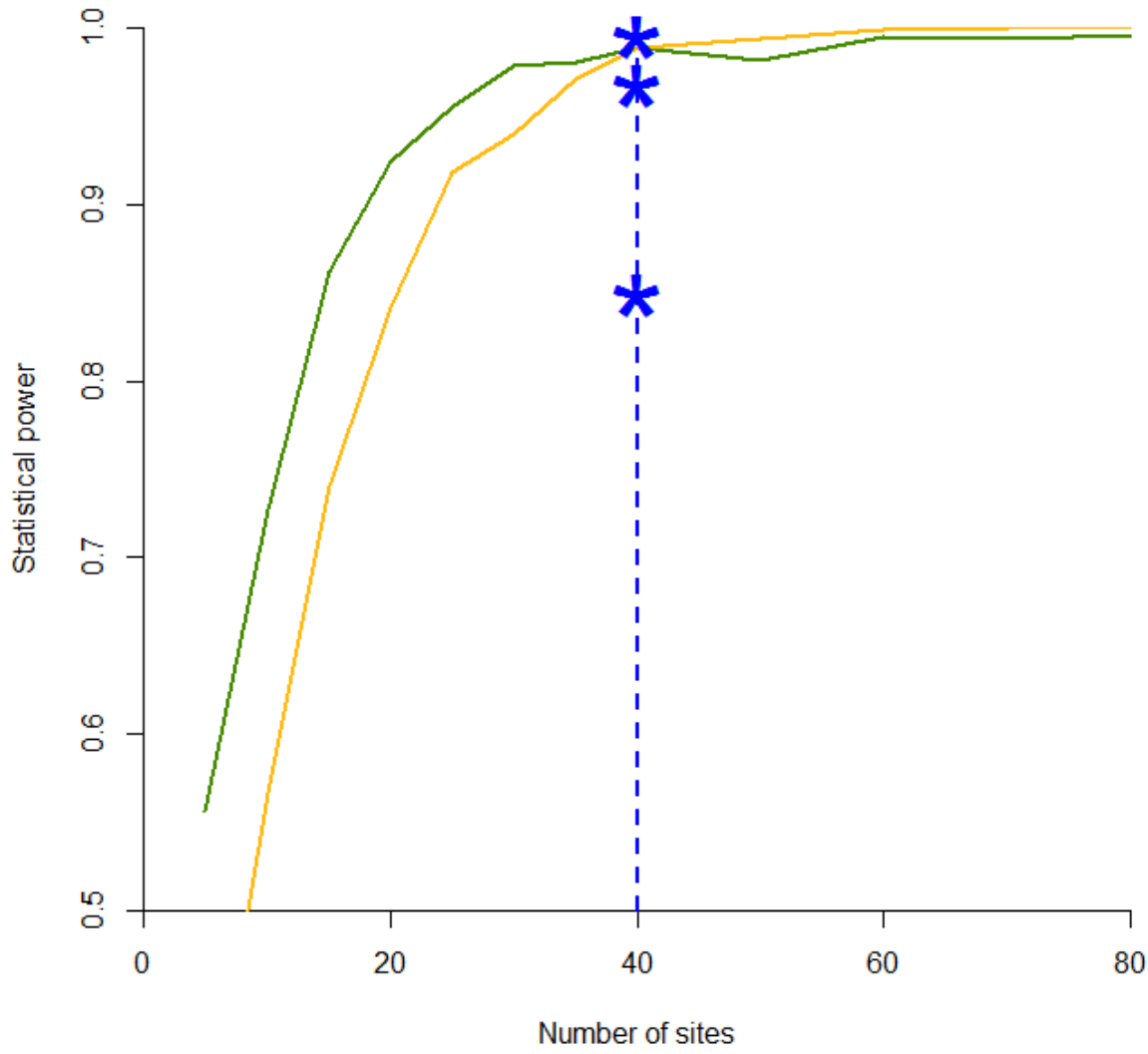

Figure E1 Illustrating the statistical power of the transect method to detect the effect of temperature on blue tit phenology, and how this varies with different numbers of sites and nestboxes operated. The green line depicts the results of (i) (see methods), i.e. how statistical power varies when 240 nestboxes are spread evenly across a variable number of sites. The yellow line depicts the results of (ii) (see methods), i.e. how statistical power varies when six nestboxes are operates at each of a variable number of sites. The blue stars depict the results of (iii) (see methods), i.e. how statistical power varies when variable numbers of nestboxes are operated at 40 sites, with the lowest star indicating the statistical power when two

nestboxes are operated per site, the middle star four nestboxes, and the highest star eight nestboxes. The blue dashed line shows how many sites were used in the real transect, 40. The statistical power of the real transect is the point at which the green, yellow and blue lines converge, 0.989.

Discussion

This analysis shows that the transect provides excellent statistical power to detect effects of the magnitude simulated (i.e. the effect of temperature on blue tit phenology). Whilst increasing sampling effort always improves statistical power, in reality this is curtailed by practical limitations including money, manpower and time, particularly when studying a transect of the magnitude operated in this thesis. These simulations demonstrate that a transect incorporating 40 sites each with six nestboxes provides a good compromise between logistics and statistical power, whereby fewer sites or nestboxes per site considerably reduces statistical power, yet more sites or nestboxes per site does not considerably increase statistical power, and the current level of statistical power is acceptable. In addition, whilst the simulations were run over one 'year', in this thesis data was recorded over three years, which would increase the statistical power to detect any genuine effect still further.

Appendix F

Field season reports and transect bird list

Section F1 2014 field season report sent out to landowners and managers and interested parties.

Dear All,

An end of season 'hello' from the nestbox researchers here at Edinburgh University. We hope that you've all had a wonderful spring and summer and we'd like to report back some preliminary results from the project this year and outline our plans for the future. Once again a huge thank you to everyone for allowing us access to your land.

At a quick glance 2014 appears to have been a wonderful success for our Blue Tits. We had 89 nesting attempts in our 180 boxes across 30 sites – an occupancy rate close to 50%, which is above expected for the first year of a nestbox scheme, especially one that extends to such high altitudes. The first egg of the year was found on the 13th April at the Foulis Estate with the last egg laid at Dornoch on the 21st May; the nesting season being over by the end of June. Overall there were 778 eggs laid at an average of 8.7/nest from which 706 chicks hatched and 616 made it through all the way to fledging, giving an amazingly high 80% fledging success rate. This may be due to favourable weather (think back to the wonderful spring during the present deluge!) and, at a glance, the tits seem to have timed their breeding efforts well to coincide with the insect peaks at each of the sites. In addition to checking the nestboxes, we recorded hourly temperatures, the timing of budburst and leafing of a sample of trees at each site alongside monitoring the invertebrate community via sticky traps and branch beating. Our motivation for doing this is to detect the effect of spring conditions on the ability of the birds to time their chick-rearing to coincide with an abundance of invertebrates.

The lowland sites, as expected, had a higher occupancy rate than the upland sites, with Fordell Firs and Ballinluig the only ones to have complete nestbox occupancy, but the upland pairs had surprisingly high success. Indeed, the 'best nest' was at Calvine, our fourth highest site, which fledged 14 youngsters and the only second brood (very rare in blue tits, particularly this far north) came from the pair at Dalnacardoch, our second highest site, in July. This incredible highland pair successfully raised 17 chicks (11 followed by 6) this year – a record for any project that I've been involved in. A table is presented at the bottom of this email should anyone be interested about how their own blue tits fared. A couple have had zero success this year, but not to worry, we think this is down to chance, as we've seen blue tits at all sites.

As for the future, we need to return to each of the sites a couple of times this autumn for a habitat survey and then we may conduct some winter trapping of blue tits at certain sites to ascertain survival. Otherwise, as far as the sites are concerned, the next field season will begin in earnest again in mid-March 2015 where we shall look forward to another successful year! In the meantime I will be in the lab analysing the samples we have collected and I hope to update you on preliminary findings prior to the next field season.

If you would like any further information or have any questions regarding our future plans, please don't hesitate to get in touch.

Best wishes,
Jack Shutt

A quick summary of the 2014 field season at each site:

Site	Boxes	Nests	Eggs	Chicks	Fledglings	Success %
Fordell Firs	6	6	55	47	34	61.8
Blairadam	6	4	42	40	38	90.5
Loch Leven	6	4	33	30	28	84.8
Glenfarg	6	5	48	46	40	83.3
Moncrieffe Hill	6	3	30	30	29	96.7
Perth	6	4	33	30	30	90.9
Stanley	6	3	35	35	35	100.0
Birnam	6	3	28	27	27	96.4
Dunkeld	6	1	6	2	0	0.0
Ballinluig	6	6	53	47	39	73.6
Killiecrankie	6	4	31	29	28	90.3
Blair Atholl	6	3	25	25	16	64.0
Calvine	6	2	16	14	14	87.5
Dalnamein	6	0	0	0	0	0.0
Dalnacardoch	6	1	11	11	11	100.0
Dalwhinnie	6	1	11	11	10	90.9
Crubenmore	6	2	17	15	11	64.7
Newtonmore	6	3	26	26	23	88.5
Insh	6	1	8	7	7	87.5
Feshiebridge	6	2	19	19	19	100.0
Aviemore	6	1	8	8	8	100.0
Carrbridge	6	2	14	12	12	85.7
Tomatin	6	0	0	0	0	0.0
Daviot	6	4	35	33	28	80.0
Munlochy	6	4	30	28	19	63.3
Foulis Estate	6	4	35	34	32	91.4
Alness	6	2	19	18	17	89.5
Delny Muir	6	5	39	31	29	74.4
Tain Pottery	6	5	31	28	17	54.8
Dornoch	6	4	30	23	15	50.0
Overall	180	89	778	706	616	79.2

Section F2 2015 field season report sent out to landowners and managers and interested parties.

Dear All,

This is an end of season 'hello' from the Blue Tit nestbox researchers here at Edinburgh University to report back some preliminary results from the project this year and outline our plans for the future. Once again a huge thank you to everyone for allowing us access to your land.

As some may remember, back in 2014 conditions were wonderful for the Blue Tits all along the transect, from Edinburgh up to Dornoch. The good spring weather and abundant caterpillars and insects at just the right time, resulted in a fantastic 80% fledging success rate. This, combined with a fairly mild winter and excellent winter food resources (it was a beechmast year) resulted in there being many more Blue Tits in the woods this spring. In fact, our occupancy rates rose from 48% in 2014 to 69% in 2015, with 160 of our 232 nestboxes having a nest.

However, I am afraid to say that is where the good news ends for the Blue Tit's 2015 breeding season. Whilst Southern Europe baked in excessive heat, Scotland and much of Northern Europe had a particularly cold and wet spring, and we even experienced snows in June. This delayed trees coming into leaf and meant much fewer insect resources were available for the birds. A quick look at our data suggests caterpillar numbers were around four times lower than in 2014. All of this took its toll on the birds.

Incredibly, the first egg of the year was once again found at nestbox 1 at the Foulis Estate near Dingwall on the 17th April, just four days later than last year, although this nest later failed. From this point however the spring progressed fairly slowly with the last egg laid on 31st May at Spinningdale and the nesting season being over by the start of July. Overall there were 1189 eggs laid at an average of 7.4/nest (slightly down from 8.7/nest in 2014) and 570 chicks fledged, giving a very poor 47.9% success rate, down from 80.6% in 2014. In addition to checking the nestboxes, we recorded hourly temperatures, the timing of budburst and leafing of a sample of trees at each site alongside monitoring the invertebrate community via sticky traps and branch beating. Our motivation for doing this is to detect how the birds time their chick-rearing to coincide with an abundance of invertebrates.

Success in 2015 seems at least partially down to habitat, with oak-dominated sites performing remarkably well and alder- and sycamore-dominated sites fledging very few birds. It may be that in cold springs these types of woodland cannot support the quantity of invertebrates necessary for successful Blue Tit breeding. The 'best nest' was once again at Calvine and fledged 10 of an impressive brood of 13 – with the father being the same individual who helped raise 2014's 'best nest' of 14 fledglings with a different female at the same site. Indeed, we had quite a few of the same adults as 2014, as well as a couple of chicks born in 2014 returning to breed. We detected no movement of birds between our sites and some even nested in the exact same nestbox as the year before, illustrating how locally faithful Blue Tits can be. A table is presented at the bottom of this report should anyone be interested about how their own blue tits fared.

As for the future, the next field season will begin in earnest again in mid-March 2016 and shall be my last on the project. We shall cross our fingers for a more successful year! In the

meantime I will be in the lab analysing the samples we have collected this spring. If you would like any further information or have any questions regarding our future plans, please don't hesitate to get in touch.

Best wishes,
Jack Shutt

A quick summary of the 2015 field season at each site:

Site	Boxes	Nests	Eggs	Chicks	Fledglings	Success %
Edinburgh	6	6	49	39	20	40.8
Rosyth	6	6	49	41	2	4.1
Fordell Firs	6	6	44	43	28	63.6
Blairadam	6	5	41	36	19	46.3
Loch Leven	6	6	33	29	22	66.7
Glenfarg	6	6	50	33	21	42.0
Bridge of Earn	6	6	63	53	19	30.2
Moncrieffe Hill	6	4	28	22	19	67.9
Perth	6	6	46	39	34	73.9
Stanley	6	6	48	41	10	20.8
Birnam	6	6	42	41	23	54.8
Dunkeld	6	3	20	14	7	35.0
Ballinluig	6	6	41	39	31	75.6
Killiecrankie I	6	4	24	20	8	33.3
Killiecrankie II	6	4	32	30	30	93.8
Blair Atholl	6	5	38	33	27	71.1
Calvine	6	2	20	20	17	85.0
Dalnamein	6	2	20	20	6	30.0
Dalnacardoch	6	2	20	19	6	30.0
Dalnaspidal	4	1	7	7	3	42.9
Dalwhinnie	6	2	20	20	5	25.0
Crubenmore	6	3	19	11	10	52.6
Newtonmore	6	4	29	28	12	41.4
Insh	6	2	11	9	0	0.0
Feshiebridge	6	5	36	36	9	25.0
Rothiemurchus	6	2	16	16	15	93.8
Aviemore	6	5	30	25	25	83.3
Avielochan	6	6	48	38	36	75.0
Carrbridge	6	4	26	23	11	42.3
Slochd Summit	6	2	16	14	4	25.0
Tomatin	6	1	9	9	3	33.3
Daviot	6	4	36	35	5	13.9
Munlochy	6	4	30	29	12	40.0
Foulis Estate	6	5	34	31	19	55.9
Alness	6	4	32	29	16	50.0
Delny Muir	6	6	42	31	24	57.1
Tain Pottery	6	4	27	24	23	85.2
Spinningdale	6	2	10	9	4	40.0
Dornoch	6	3	21	21	4	19.0

Section F3 2016 field season report sent out to landowners and managers and interested parties.

Dear All,

'Hello' from the Blue Tit nestbox researchers here at Edinburgh University at the end of our third field season, and the final one of my PhD. As many of you know we run a 200-mile transect across Scotland from Edinburgh in the south to Dornoch in the north and visit each site every other day throughout spring to study blue tit productivity in our woodlands and the effect of spring temperatures on seasonal timings. We could not do this without your help so once again a huge thank you to everyone for allowing us access to your land. In this report we would like to share with you a quick summary of the blue tit nesting outcomes from the project this year.

As some may remember, back in 2014 conditions were wonderful for the Blue Tits (and the humans!) all along the transect, with fantastic spring weather (now a distant memory!) and abundant caterpillars and insects at just the right time, resulting in a whopping 80% fledging success rate. 2015, on the other hand, was exceptionally poor for the birds, with persistent cold rain reducing the food supply and therefore the fledging success to a meagre 48%.

This year calm was restored, with an intermediate year splitting the difference between these exceptional years in almost every way. Our occupancy rates remained steady at 62%, with 146 of our 237 nestboxes having a nesting attempt, not too adversely affected by the appalling fledging success last year because of a mild winter where the adults survived in good numbers. We found very few young birds from last year breeding this year though, reflecting the poor fledging success. The first egg of the year was on the 19th April at sea level at Alness, two days later than last year with the last egg being laid on the 7th June at high elevation in the Cairngorms at Dalnacardoch a full 7 weeks later, and the nesting season is all but over by the start of July.

Overall there were 1173 eggs laid at an average of 8.0/nest (8.7 in 2014, 7.4 in 2015) and 780 chicks fledged, giving a 67% success rate (80% in 2014, 48% in 2015). This meant that 5.3 chicks fledged per nesting attempt (6.9 in 2014, 3.7 in 2015). Our most productive nests this year both fledged 11 chicks, one at Avielochan and the other at Dalnacardoch, which this year experienced a very localised and incredible population explosion of winter moth caterpillars unlike any we've seen throughout the three years; they chewed through so many leaves it looked like mid-winter in June and was fantastic for both the moth and bird populations. Unlike in 2015 where habitat played a major part in determining success, productivity was more uniform across the sites this year. A table is presented on the next page of this report should anyone be interested about how their own blue tits fared.

In addition to checking the nestboxes, we recorded hourly temperatures, the timing of budburst and leafing of a sample of trees at each site alongside monitoring the invertebrate community via sticky traps and branch beating. Our motivation for doing this is to detect how the birds time their chick-rearing to coincide with an abundance of invertebrates, and which caterpillar species are available.

As for the future, I will now begin analysing and writing up my PhD in earnest and will contact you again with a summary of the key findings once it is complete. My supervisor (Ally Phillimore – albert.phillimore@ed.ac.uk) is in the process of applying for funding to continue the project. He will be in touch in the coming months to ask whether you mind the project continuing at your site or whether you'd prefer the next boxes to be removed. If you would like any further information or have any questions regarding our future plans, please don't hesitate to get in touch.

Best wishes and thanks again for your support of the project,
Jack Shutt

A quick summary of the 2016 field season at each site, from south to north:

Site	Boxes	Nests	Eggs	Chicks	Fledglings	**Success %**
Edinburgh	6	6	50	46	32	**64.0**
Rosyth	6	5	40	37	28	**70.0**
Fordell Firs	6	6	54	53	50	**92.6**
Blairadam	6	5	38	34	33	**86.8**
Loch Leven	6	3	22	21	15	**68.2**
Glenfarg	6	6	49	46	37	**75.5**
Bridge of Earn	6	6	50	47	47	**94.0**
Moncrieffe Hill	6	4	38	38	18	**47.4**
Perth	5	4	25	22	21	**84.0**
Stanley	6	6	44	41	32	**72.7**
Birnam	6	5	34	30	14	**41.2**
Dunkeld	6	2	16	16	3	**18.8**
Ballinluig	6	6	54	50	22	**40.7**
Killiecrankie I	6	3	24	24	6	**25.0**
Killiecrankie II	6	5	43	41	25	**58.1**
Blair Atholl	6	3	18	17	9	**50.0**
Calvine	6	2	21	21	8	**38.1**
Dalnamein	6	2	20	17	13	**65.0**
Dalnacardoch	6	2	18	18	18	**100.0**
Dalnaspidal	4	1	8	6	0	**0.0**
Dalwhinnie	6	1	10	10	10	**100.0**
Crubenmore	6	2	18	18	15	**83.3**
Newtonmore	6	4	29	29	20	**69.0**
Insh	6	1	9	9	9	**100.0**
Feshiebridge	6	3	26	17	16	**61.5**
Rothiemurchus	6	3	22	20	20	**90.9**
Aviemore	6	3	24	16	15	**62.5**
Avielochan	6	5	44	42	42	**95.5**
Carrbridge	6	2	14	14	4	**28.6**
Slochd Summit	6	3	25	24	15	**60.0**
Tomatin	6	1	8	8	5	**62.5**
Daviot	6	3	26	25	12	**46.2**
Artafallie	6	6	49	33	20	**40.8**
Munlochy	6	4	34	26	24	**70.6**
Foulis Estate	6	4	28	26	23	**82.1**
Alness	6	3	22	22	16	**72.7**
Delny Muir	6	6	43	39	39	**90.7**
Tain Pottery	6	6	47	43	30	**63.8**
Spinningdale	6	2	15	14	10	**66.7**
Dornoch	6	2	14	14	4	**28.6**
Overall	237	146	1173	1074	780	**66.5**

Section F4 Transect bird list.

1	Mute Swan	49	Dunlin	97	Song Thrush
2	Whooper Swan	50	Jack Snipe	98	Mistle Thrush
3	Pink-footed Goose	51	Snipe	99	Fieldfare
4	Greylag Goose	52	Woodcock	100	Grasshopper Warbler
5	Canada Goose	53	Curlew	101	Sedge Warbler
6	Barnacle Goose	54	Common Sandpiper	102	Icterine Warbler
7	Shelduck	55	Greenshank	103	Blackcap
8	Wigeon	56	Redshank	104	Garden Warbler
9	Gadwall	57	Herring Gull	105	Whitethroat
10	Teal	58	Common Gull	106	Wood Warbler
11	Mallard	59	Ring-billed Gull	107	Chiffchaff
12	Shoveler	60	Lesser Black-backed Gull	108	Willow Warbler
13	Pochard	61	Great Black-backed Gull	109	Spotted Flycatcher
14	Tufted Duck	62	Black-headed Gull	110	Pied Flycatcher
15	Eider	63	Common Tern	111	Goldcrest
16	Goldeneye	64	Arctic Tern	112	Long-tailed Tit
17	Red-breasted Merganser	65	Sandwich Tern	113	Blue Tit
18	Goosander	66	Woodpigeon	114	Great Tit
19	Smew	67	Stock Dove	115	Coal Tit
20	Red Grouse	68	Collared Dove	116	Crested Tit
21	Ptarmigan	69	Rock Dove	117	Treecreeper
22	Black Grouse	70	Cuckoo	118	Nuthatch
23	Red-legged Partridge	71	Tawny Owl	119	Jay
24	Grey Partridge	72	Long-eared Owl	120	Magpie
25	Pheasant	73	Kingfisher	121	Jackdaw
26	Little Grebe	74	Green Woodpecker	122	Rook
27	Great Crested Grebe	75	Great Spotted Woodpecker	123	Carrion Crow
28	Gannet	76	Skylark	124	Hooded Crow
29	Cormorant	77	Swift	125	Raven
30	Grey Heron	78	Sand Martin	126	Starling
31	Red Kite	79	House Martin	127	House Sparrow
32	Honey Buzzard	80	Swallow	128	Tree Sparrow
33	Buzzard	81	Tree Pipit	129	Chaffinch
34	Hen Harrier	82	Meadow Pipit	130	Brambling
35	Goshawk	83	Pied Wagtail	131	Greenfinch
36	Sparrowhawk	84	Grey Wagtail	132	Goldfinch
37	Golden Eagle	85	Waxwing	133	Siskin
38	White-tailed Eagle	86	Dipper	134	Linnet
39	Osprey	87	Wren	135	Twite
40	Kestrel	88	Dunnock	136	Lesser Redpoll
41	Merlin	89	Robin	137	Mealy Redpoll
42	Peregrine	90	Redstart	138	Common Crossbill
43	Water Rail	91	Whinchat	139	Scottish Crossbill
44	Moorhen	92	Stonechat	140	Bullfinch
45	Coot	93	Wheatear	141	Yellowhammer
46	Oystercatcher	94	Redwing	142	Reed Bunting
47	Golden Plover	95	Ring Ouzel		
48	Lapwing	96	Blackbird		